ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE
FISCAL YEAR 1974
VOLUME I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

Library, Acquisitions Unit
National Institutes of Health
Building 10
Bethesda, Maryland 20014

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

Fiscal Year 1974

Volume I

RC
346
N2775
1974
v.1

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

ANNUAL REPORT

July 1, 1973 through June 30, 1974

OFFICE OF THE DIRECTOR

The Director's Report 1-a

INTRAMURAL RESEARCH

Table of Organization 1-b

Scientific Director's Report 1-c

Acting Clinical Director's Report 1-d

Office of the Director of Intramural Research

NDS(I)-66 ODIR 1460
Speech habilitation of the congenitally deaf--
Fundamental problems and suggested directions 1-e

NDS(I)-68 ODIR 1554
Pure and applied mathematics, with special attention to mathematical models of sensory systems and of research institutions 3-e

NDS(I)-70 ODIR 1789
The Physical Senses 5-e

Medical Neurology Branch, Summary Report 1-f

NDS(I)-62 MN/OC 915(c)
Histochemistry applied to the study of neurologic disease 9-f

NDS(I)-62 MN/OC 917(c)
Biochemistry applied to the study of neurologic disease 15-f

NDS(I)-63 MN/OC 1034(c)
Myopathies 21-f

NDS(I)-63 MN/OC 1037(c)
Tissue culture applied to the study of human neurologic disease 27-f

NDS(I)-63 MN/OC 1039(c)
Amyotrophic lateral sclerosis (ALS) and other lower motor neuron diseases, including peripheral neuropathies and spinocerebellar ataxias 31-f

NDS(I)-65 MN/OC 1189(c)
Episodic weakness 41-f

NDS(I)-65 MN/OC 1190(c)
Myasthenia gravis (MG) 43-f

NDS(I)-65 MN/OC 1191(c)
Immunological abnormalities of neurologic diseases 49-f

NDS(I)-65 MN/OC 1192(c)
Electromicroscopic studies of skeletal muscle and neurons 53-f

NDS(I)-65 MN/OC 1193(c)
Radioautography applied to the study of neurologic disease 59-f

NDS(I)-69 MN/OC 1728(c)
Brain tumor 63-f

NDS(I)-70 MN/OC 1792(c)
Electrophysiology applied to the study of neurologic disease 67-f

NDS(I)-62 MN/AP 926(c)
Electromechanical coupling in muscle and drug activity 71-f

NDS(I)-63 MN/AP 1049(c)
Mechanical properties of muscle in relation to drug action 73-f

NDS(I)-63 MN/AP 1050(c)
Functional activity and properties of striated muscle 75-f

NDS(I)-67 MN/AP 1416(c)
A pharmacological and toxicological study of neurotoxic venoms 77-f

NDS(I)-70 MN/AP 1809(c)
Impaired transfer of information between nerve and muscle 79-f

Surgical Neurology Branch, Summary Report 1-g

NDS(I)-54 SN/OC 100(c)
Epileptogenic mechanisms in the brain of man and other primates 7-g

NDS(I)-55 SN/OC 200(c)
Involuntary movements 13-g

NDS(I)-56 SN/OC 304(c)
Effect of lesions upon the function and structure of the human central nervous system 15-g

NDS(I)-62 SN/OC 907(c)
Trauma to the nervous system 19-g

NDS(I)-62 SN/OC 913(c)
Single cell discharges from various nervous structures and their functional organization in particular reference to somatosensory activity in man 23-g

NDS(I)-63 SN/OC 1025(c)
Tumors of the nervous system 25-g

NDS(I)-63 SN/NR 1047(c)
Isotope-ventriculography and isotope-cisternography 29-g

NDS(I)-65 SN/NR 1195(c)
Radiographic and radioisotopic angiography of the spinal cord 33-g

NDS(I)-66 SN/OC 1245(c)
EEG learning correlates using scalp and intracranial depth electrodes 39-g

NDS(I)-67 SN/NR 1413(c)
Radioactive scanning tomography of the brain 41-g

NDS(I)-67 SN/OC 1417(c)
Changes in the physiological properties of brain tissue at low temperature and in other pathological conditions 45-g

NDS(I)-67 SN/OC 1424(c)
Response modulation by the limbic system in man: neuropsychological and physiological changes with amygdaloid and cingulate lesions 47-g

NDS(I)-68 SN/OC 1526(c)
The epileptic neurons and their recurrent axon collaterals 49-g

NDS(I)-68 SN/OC 1527(c)
Physiological mechanism of motor function in the cat 51-g

NDS(I)-68 SN/NR 1654(c)
Experimental spinal cord angiography 53-g

NDS(I)-68 SN/OC 1658(c)
Hemispheric development and specialization of intellectual functions 55-g

NDS(I)-69 SN/NR 1791(c)
Angiography of cerebral venous obstructive disease 59-g

NDS(I)-71 SN/NR 1866(c)
Studies on cerebral blood flow in normal and infarcted brain by radiographic and radioisotopic methods 61-g

NDS(I)-73 SN/NR 2008(c)
Supine myelography 65-g

NDS(I)-73 SN/OC 2009(c)
Neurological basis of acupuncture-analgesia 69-g

NDS(I)-73 SN/OC 2010(c)
Mechanisms of pain 71-g

NDS(I)-74 SN/NR 2072(c)
Blood-brain barrier and radiographic contrast media 73-g

NDS(I)-74 SN/NR 2073(c)
Computerized axial tomography of hindbrain and spinal cord 75-g

Central Nervous Systems Studies Laboratory, Summary 1-h

NDS(I)-65 CNSS 969
Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections 11-h

NDS(I)-65 CNSS 1282
Neurobiology of Population Isolates: Study of child growth and development, behavior and learning, and disease patterns in primitive cultures 39-h

Branch of Electroencephalography and Clinical Neurophysiology, Summary Report 1-i

NDS(I)-72 EEG/CN 1939a
Fluorometric monitoring of intracellular redox changes in the intact cat brain 7-i

NDS(I)-72 EEG/CN 1939b
Fluorometric monitoring of intracellular redox changes in human cerebral cortex 9-i

NDS(I)-72 EEG/CN 1940(c)
Surgery of temporal lobe epilepsy. Pre- and postoperative EEG and clinical data 11-i

NDS(I)-73 EEG/CN 2011
Topographical analysis of cortical redox changes surrounding epileptic foci 15-i

NDS(I)-73 EEG/CN 2012
Development of abnormal neuronal responses during the evolution of an epileptic focus in feline visual cortex 17-i

NDS(I)-73 EEG/CN 2013
The effects of epileptogenic agents upon the single unit responses in the cerebral cortex 19-i

NDS(I)-73 EEG/CN 2014
Clinical seizure patterns in patients with occipital epileptogenic foci 21-i

NDS(I)-74 EEG/CN 2094
Use of a television fluorometer to study simultaneous oxidative and K^+ changes in primary and dependent mirror epileptogenic foci 23-i

NDS(I)-74 EEG/CN 2095
Simultaneous monitoring of changes in extracellular K^+ concentration and oxidative metabolism in intact cat hippocampus and cerebral cortex 25-i

NDS(I)-74 EEG/CN 2096
Effects of electrical stimulation of the ponto-mesencephalic reticular formation on the electrical and metabolic activity of cortical areas in the acute cat 27-i

Developmental and Metabolic Neurology Branch,
Summary Report 1-j

NDS(I)-60 DMNB/CIT 706(c)
Clinical, genetic, biochemical and pathological studies of progressive cerebral degenerations, mental retardation and birth defects; with emphasis on familial occurrence 5-j

NDS(I)-61 DMN/EG 815
Metabolism of complex lipids of nervous tissue, studies on Gaucher's disease, Niemann-Pick disease, Fabry's disease, Krabbe's disease and Metachromatic Leukodystrophy 11-j

NDS(I)-61 DMN/EG 816
Structural and metabolic studies of gangliosides in normal humans and patients with Tay-Sachs disease 15-j

NDS(I)-63 DMN/CIT 1026(c)
Clinical and biochemical studies on epilepsy 17-j

NDS(I)-66 DMN/EG 1309
Studies on the metabolism of sphingolipids in tumor tissues 21-j

NDS(I)-67 DMN/NM 1457
The chemical synthesis of radioactive sphingolipids 25-j

NDS(I)-68 DMN/CIT 1523(c)
Neurological, general medical and biochemical aspects of Hurler, Hunter, Sanfilippo and Scheie diseases; basic pathogenesis, enzymology and classification criteria 27-j

NDS(I)-69 DMN/CIT 1655
Studies of the composition and biosynthesis of cerebral proteins in experimental animals and man 31-j

NDS(I)-70 DMN/EG 1808
Glycoprotein metabolism in the myelinating central nervous system 35-j

NDS(I)-73 DMN/CIT 2024(c)
Study of plasma partitioned lipids, lipoprotein and blood viscosity in patients with transient or definitive strokes and in family members with Hyperlipidemia 41-j

NDS(I)-74 DMN/CIT 2103
Effects of intravenous infusion of Ceramidetrihexosidase in patients with Fabry's disease and Glucocerebrosidase in patients with Gaucher's disease; a therapeutic intent 45-j

Laboratory of Neuropathology and Neuroanatomical Sciences, Summary Report 1-k

NDS(I)-71 LNNS/CNC 1884
Brain glycogen synthetase 7-k

NDS(I)-71 LNNS/CNC 1885
The kinetics of brain glycogen 9-k

NDS(I)-72 LNNS/CNC 1942
The role of adenosine 3',5' cyclic monophosphate (cyclic AMP) and guanosine 3',5' cyclic monophosphate (cyclic GMP) in the central nervous system 13-k

NDS(I)-73 LNNS/CNC 2006
Regulation of metabolism in glioma and neuroblastoma cell lines 17-k

NDS(I)-73 LNNS/CNC 2007
Regulation of the NAD+/NADH ratio in normal and virally-transformed tumor cell lines 19-k

NDS(I)-70 LNNS/CN 1802
Study of living nerve and muscle fibers by differential interference microscopy 21-k

NDS(I)-70 LNNS/CN 1803
Electron microscopic study of axon-Schwann cell relationships prior to myelination in peripheral nerves 23-k

NDS(I)-73 LNNS/CN 1995
Morphological studies of CNS demyelination and remyelination in model systems 25-k

NDS(I)-73 LNNS/CN 1996
Myelin membrane structure - morphological comparisons in intact CNS tissue and subcellular brain fractions 29-k

NDS(I)-74 LNNS/CN 2082
Human muscle spindles: Fine structure of the IA sensory endings 31-k

NDS(I)-73 LNNS/CP 1997
Cerebrovascular reactions to ischemia in the Mongolian gerbil 33-k

NDS(I)-73 LNNS/CP 1998
Central biogenic amines in edema and ischemia 35-k

NDS(I)-74 LNNS/CP 2093
Development of pressure gradients within brain tissue during the formation of vasogenic brain edema 39-k

NDS(I)-74 LNNS/CP 2104
Evaluation of the "no-reflow" (NR) phenomenon in Mongolian gerbils 41-k

NDS(I)-74 LNNS/CP 2105
Behavior of the blood-brain barrier and the development of edema in the cerebral ischemia 45-k

NDS(I)-64 LNNS/ENP 1066
The specificity of neuronal changes in cerebral infarcts 49-k

NDS(I)-67 LNNS/ENP 1449
The effect of cortisone on the retrograde neuronal changes 51-k

NDS(I)-69 LNNS/ENP 1676
A comparative study of the area postrema 53-k

NDS(I)-73 LNNS/ENP 2002
Mast cells in the brain 55-k

NDS(I)-73 LNNS/ENP 2003
The thalamo-choroidal body 57-k

NDS(I)-73 LNNS/ENP 2004
The pathogenesis of myelopathies 59-k

NDS(I)-73 LNNS/ENP 2005
Heredopathia atactica polyneuritiformis (Refsum's disease) 61-k

NDS(I)-65 LNNS/FN 1231
Organization of synaptic connections in the mammalian brain 63-k

NDS(I)-67 LNNS/FN 1442
Permeability of cellular layers in the vertebrate nervous system 67-k

NDS(I)-69 LNNS/FN 1684
Structure and function of close intercellular (gap) junctions 71-k

NDS(I)-71 LNNS/FN 1881
Structural basis of synaptic transmission 73-k

NDS(I)-71 LNNS/NC 1879
Chemical changes in brain of transplacentally methylazoxymethanol (MAM) induced microencephaly in rats 77-k

NDS(I)-71 LNNS/NC 1880
Hyperosmolar and $HgCl_2$ effect on the brain uptake of ^{14}C glucose analogues 79-k

NDS(I)-73 LNNS/NC 1999
Brain uptake of ^{14}C 3-O-methyl-D-glucose and ^{14}C sucrose in ischemic gerbils 81-k

NDS(I)-73 LNNS/NC 2000
Brain edema in cerebral ischemia of gerbils 85-k

NDS(I)-73 LNNS/NC 2001
The effect of oxygen saturation and pCO_2 on glucose transport in the rabbit brain 87-k

NDS(I)-74 LNNS/NC 2083
Glucose transport in cerebellar cultures 89-k

NDS(I)-74 LNNS/NC 2084
The growth and morphologic properties of cerebellar and meningeal capillaries 91-k

NDS(I)-74 LNNS/NC 2085
Ultrastructural changes in cerebral ischemia of gerbils 93-k

NDS(I)-67 LNNS/NC 1443
The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents 95-k

NDS(I)-68 LNNS/NC 1587
A blood-brain barrier to peroxidase in the normal and injured brain of elasmobranchs 97-k

NDS(I)-69 LNNS/NC 1678
Distribution of exogenous proteins in brain tumors 99-k

NDS(I)-69 LNNS/NC 1805
Structural changes within the membranes of stimulated smooth muscle and neurosecretory cells 101-k

NDS(I)-72 LNNS/NC 1965
The effect of biogenic amines on blood-brain barrier to peroxidase 103-k

NDS(I)-74 LNNS/NC 2086
Regeneration in vertebrate and invertebrate nerves 105-k

Laboratory of Neural Control, Summary Report 1-1

NDS(I)-69 LNLC/OC 1686
Motor control system in the spinal cord 7-1

NDS(I)-69 LNLC/OC 1687
Techniques for making connections with the nervous system 13-1

NDS(I)-69 LNLC/OC 1688
Cortical mechanisms of voluntary motor control 17-1

NDS(I)-73 LNLC/OC 2015
Neural prosthesis program 21-1

NDS(I)-74 LNLC/OC 2078
Input-output pathways of dorsal root ganglion cells 25-1

NDS(I)-74 LNLC/OC 2079
Models of neural interactions 27-1

NDS(I)-74 LNLC/OC 2080
Neuron activity during locomotion 31-1

Laboratory of Neurophysiology, Summary Report 1-m

NDS(I)-65 LNP/SP 1239
Photoreceptors in the Limulus eyes 3-m

NDS(I)-69 LNP/SP 1690
Rapid scanning microspectrophotometry in visual cells 7-m

NDS(I)-73 LNP/SP 2019
Electrophysiology of simple cellular systems 9-m

NDS(I)-69 LNP/CB 1659
Synaptic contacts of visual cells 13-m

NDS(I)-73 LNP/CB 2016
Synaptic organization of the inner plexiform layer of the larval salamander retina 15-m

NDS(I)-71 LNP/GP 1889
Studies of agents affecting cell proliferation and differentiation in the crystalline lens 17-m

NDS(I)-72 LNP/GP 1943
Spontaneous retinal degeneration in Osborne-Mendel rats 21-m

NDS(I)-72 LNP/GP 1944
Organization of a molluscan central nervous system 25-m

NDS(I)-72 LNP/GP 1945
Studies of neural organization of the vertebrate retina 29-m

NDS(I)-73 LNP/GP 2017
Mechano-Transduction in invertebrate hair cells 33-m

NDS(I)-73 LNP/GP 2018
The ionic and pharmacologic basis of the inhibitory interaction between photoreceptors of a simple visual system 37-m

NDS(I)-71 LNP/NI 1892
Comparative physiology of invertebrate photoreceptors 39-m

NDS(I)-71 LNP/NI 1895
Microspectrophotometric studies of vertebrate photoreceptors 41-m

Laboratory of Biophysics, Summary Report 1-n

NDS(I)-62 LB/OC 935
Ionic permeabilities of excitable membranes. Electrical experiments and analyses 7-n

NDS(I)-62 LB/OC 936
Ionic permeabilities of nerve membranes: theoretical investigations 9-n

NDS(I)-62 LB/OC 939
Ionic permeabilities of the squid giant axon membrane, electrical experiments and analyses with alterations of environments 11-n

NDS(I)-62 LB/OC 940
Ionic permeabilities of excitable membranes: effects of chemical agents 13-n

NDS(I)-65 LB/OC 1240
Molecular biophysics - physical properties of membranes and simple membrane-like systems 15-n

NDS(I)-72 LB/OC 1947
Cytoplasmic water structure 17-n

NDS(I)-72 LB/OC 1948
Electrical properties of small patches of excitable membrane 19-n

NDS(I)-72 LB/OC 1950
Rapid analysis of excitable membranes by means of computer voltage clamp 21-n

NDS(I)-72 LB/OC 1966
Function and structure of membrane ionic channels obtained from studies involving ion interactions with the membranes of giant axons 25-n

NDS(I)-72 LB/OC 1967
Electrical fluctuations of excitable membranes 27-n

NDS(I)-74 LB/OC 2087
Function and structure of ionic channels: ion gating mechanisms 29-n

NDS(I)-74 LB/OC 2088
Function and structure of membrane ionic channels: pharmacology and ionic selectivity 33-n

NDS(I)-74 LB/OC 2089
Electrical fluctuations in excitable cells 37-n

NDS(I)-74 LB/OC 2090
Lipid bilayer membranes: simulation and molecular description of excitability 39-n

NDS(I)-74 LB/OC 2091
Mathematical modeling 43-n

NDS(I)-74 LB/OC 2092
Subcellular and extracellular structures associated with nerve and muscle 49-n

Laboratory of Experimental Neurology, Summary Report 1-o

NDS(I)-69 LEN/OC 1693
Functional and structural alterations following X-irradiation of the cerebral cortex of the monkey 7-o

NDS(I)-69 LEN/OC 1694
Ontogeny of focal seizures 11-o

NDS(I)-71 LEN/OC 1897
Regional blood flow studies 15-o

NDS(I)-72 LEN/OC 1952
Vietnam Registry, analysis of yield 19-o

Laboratory of Neurochemistry, Summary Report 1-p

NDS(I)-61 LNC/AAC 810
Metabolism and function of free and protein-bound amino acids in neural tissues 5-p

NDS(I)-61 LNC/AAC 811
Electrolytes and energy metabolism in cerebral tissues 7-p

NDS(I)-74 LNC/AAC 2076
Studies on trophic substances of nerve 9-p

NDS(I)-74 LNC/AAC 2077
Physiological and biochemical correlates of cation balance in brain 11-p

NDS(I)-61 LNC/EC 813
Enzymological aspects of neural function 13-p

NDS(I)-74 LNC/EC 2075
Biochemical regulation in astrocytes 19-p

NDS(I)-63 LNC/NDR 1054
Trophic functions of the peripheral nervous system 21-p

NDS(I)-66 LNC/NDR 1303
Elucidation of factors influencing (regulating) the characteristics of skeletal muscle sensori-motor components as revealed by histochemical and other analysis 23-p

NDS(I)-68 LNC/NDR 1586
Trophic function of transplanted neurons: Demonstr-tion of neurotrophic capacity of homografted neurons in immunologically tolerant recipients 27-p

NDS(I)-74 LNC/NDR 2074
Regeneration in the central nervous system 31-p

NDS(I)-67 LNC/PM 1480
Metabolism of neurohumoral transmitter substances in marine animals 33-p

NDS(I)-67 LNC/PM 1481
Studies on the composition and metabolism of cellular membranes 37-p

Laboratory of Perinatal Physiology, Summary Report 1-q

NDS(I)-66 LPP 1388
Perinatal asphyxia and its CNS consequences 7-q

NDS(I)-67 LPP 1464
Biochemistry of the recovering rhesus monkey from various different states of energy deprivation 9-q

NDS(I)-69 LPP 1699
Functions of forebrain commissures in learning 13-q

NDS(I)-73 LPP 2020
Pathophysiology of brain injury produced by cardiovascular and pulmonary disorders 15-q

NDS(I)-73 LPP 2021
Short- and long-term behavioral deficits produced by dysergic brain disease 19-q

NDS(I)-73 LPP 2022
Neural mechanisms underlying social behavior and emotion in the rhesus monkey 21-q

NDS(I)-74 LPP 2081
Experimental production of cerebral malformations by use of teratogenic agents or severe asphyxia during pregnancy 23-q

Laboratory of Molecular Biology, Summary Report 1-r

NDS(I)-65 LMB/DB 1244
Control mechanisms and differentiation 7-r

NDS(I)-71 LMB/DB 1886
Developmental cytology 11-r

NDS(I)-72 LMB/DB 1962
Mechanism and control of membrane transport 15-r

NDS(I)-72 LMB/DB 1963
Genetic regulation in human diploid tissue culture 19-r

NDS(I)-65 LMB/MV 1208
Ribonucleic acid and the regulation of cellular metabolism 23-r

NDS(I)-73 LMB/MV 2026
Regulation of viral nucleic acids synthesis in animal cells 27-r

Laboratory of Neuro-otolaryngology, Summary Report 1-rr

COLLABORATIVE AND FIELD RESEARCH

Associate Director's Report 1-s

Section on Communicative Disorders, Summary 1-t

NDS(CF)-74 CD 2102
Temporal and masking phenomena in persons with sensorineural hearing loss 13-t

Biomedical Engineering and Instrumentation Section, Summary Report 1-u

NDS(CF)-73 BEI 2047
Biomedical engineering and instrumentation studies 13-u

Applied Neurologic Research Branch, Summary Report 1-v

NDS(CF)-71 AN 1932
Long-term electroencephalographic telemetry of patients with absence (petit mal) seizures 31-v

NDS(CF)-71 AN 1933
Quantitation of clinical manifestations of spike-wave activity by a reaction time method 33-v

NDS(CF)-72 AN 2097
Diagnostic value of prolonged telemetered EEG in epilepsy 35-v

NDS(CF)-74 AN 2098
The monaural auditory evoked potential as a measure of anticonvulsant drug effect on brain function 37-v

NDS(CF)-74 AN 2099
Pharmacokinetics of Ethotoin in epileptic patients 39-v

NDS(CF)-74 AN 2100
Analysis of KÖ 1173 (Mexiletine) by electron-capture-gas-liquid chromatography 41-v

NDS(CF)-74 AN 2101
Anticonvulsant activity of Mephenytoin in relationship to its metabolism to Nirvanol 43-v

NDS(CF)-73 AN 2051
Experimental models laboratory - head injury and stroke 45-v

NDS(CF)-74 AN 2118
Stroke models in the primate--an embolic technique for intrinsic segmental occlusion of the middle cerebral artery 47-v

NDS(CF)-74 AN 2119
Natural history of experimental cerebral infarction in conscious monkeys 49-v

NDS(CF)-74 AN 2120
Evaluation of the role of barbiturate in treating acute cerebral infarction 51-v

Epidemiology Branch, Summary Report 1-w

NDS(CF)-55 E 201
Studies on amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (ALS-PD) 19-w

NDS(CF)-63 E 1103
Neurological diseases other than ALS/PD on Guam 25-w

NDS(CF)-66 E 1319
A search for autoimmune mechanisms in the pathogenesis of chronic neurological diseases by the use of peripheral lymphocytes 27-w

NDS(CF)-67 E 1487
Genetic analysis of family data on Guam ALS and PD cases 29-w

NDS(CF)-67 E 1488
Serological studies of common viruses in cases of multiple sclerosis (MS) and controls 33-w

NDS(CF)-67 E 1490
Development of an *in vitro* serum cytotoxicity assay for amyotrophic lateral sclerosis 37-w

NDS(CF)-67 E 1496
Sequelae of CNS diseases in childhood and perinatal period 39-w

NDS(CF)-68 E 1594
Phenothiazine-induced neurological effects: A study among twins 41-w

NDS(CF)-68 E 1597
Neurologic diseases in the Trust Territories and other Pacific areas 43-w

NDS(CF)-68 E 1598
Epilepsy on Guam 45-w

NDS(CF)-68 E 1605
Phenothiazine-induced parkinsonism in white and Negro patients with nonorganic psychoses 47-w

NDS(CF)-69 E 1774
Neurologic signs and symptoms associated with malabsorption 49-w

NDS(CF)-69 E 1777
ALS among non-Chamorros after residence on Guam 51-w

NDS(CF)-69 E 1779
The epidemiology of motor neuron disease in the United States 53-w

NDS(CF)-70 E 1832
Serologic responses of multiple sclerosis patients and controls to a virus isolated from a multiple sclerosis case 55-w

NDS(CF)-70 E 1833
Immune mechanisms in chronic and congenital viral infections 57-w

NDS(CF)-70 E 1835
Epidemiologic and immunologic study of families of subacute sclerosing panencephalitis patients and families of matched controls 59-w

NDS(CF)-70 E 1836
Epidemiologic factors in Creutzfeldt-Jacob disease 61-w

NDS(CF)-70 E 1837
Multiple sclerosis distribution and patterns 63-w

NDS(CF)-70 E 1838
Study of the incidence of Parkinson's disease among offspring of Parkinsonians 65-w

NDS(CF)-70 E 1839
Studies on the relationship of chromosomal abnormalities and certain viral infections in mice. (Alternate title: Effects of viruses on mitosis and meiosis in the mouse.) 67-w

NDS(CF)-70 E 1843
Causes of death among siblings of MS and ALS patients 69-w

NDS(CF)-70 E 1844
Amyotrophic lateral sclerosis in veterans 71-w

NDS(CF)-70 E 1845
Natural history of Parkinson's disease and the effect of L-Dopa 73-w

NDS(CF)-71 E 1917
Twin study of multiple sclerosis: An epidemiologic inquiry 75-w

NDS(CF)-71 E 1920
A study of pregnancies among nurses; high risk pregnancies with possible viruses exposure 77-w

NDS(CF)-71 E 1921
A search for measles virus nucleic acid in cells of patients with multiple sclerosis (MS) 79-w

NDS(CF)-71 E 1922
Search for a negative DNA strand in cells infected with the minute virus of mice (MVM) 81-w

NDS(CF)-71 E 1923
Development of in vitro cellular immune tests for viral antigens 83-w

NDS(CF)-71 E 1924
Biochemical and pharmacologic studies in torsion dystonia 85-w

NDS(CF)-71 E 1925
Intelligence in Israeli patients with torsion dystonia 89-w

NDS(CF)-71 E 1926
The difficulty of diagnosing acoustic neuroma in young patients 91-w

NDS(CF)-71 E 1927
Clinical, genetic, biochemical and pathologic studies in hereditary early onset acoustic neuroma 93-w

NDS(CF)-71 E 1928
Biochemical and chromosome studies in retinoblastoma 95-w

NDS(CF)-71 E 1930
Von Hippel-Lindau syndrome: Clinical, genetic and biochemical aspects 97-w

NDS(CF)-72 E 1977
Selected genetic and clinical aspects of Huntington's disease 99-w

NDS(CF)-72 E 1980
Development and application of methods for the study of the minute virus of mice (MVM) 101-w

NDS(CF)-73 E 2045
Epidemiology of polymyositis in children 103-w

NDS(CF)-73 E 2046
Clinical and genetic study of the hereditary epilepsies 105-w

NDS(CF)-74 E 2113
Side effects of L-Dopa on pre-pubital patients with dystonia 107-w

NDS(CF)-74 E 2114
The study of familial Parkinson's disease 109-w

NDS(CF)-74 E 2115
Familial amyotrophic lateral sclerosis: Clinical, pathologic and genetic study 111-w

NDS(CF)-74 E 2116
Nucleic acid studies on selected oncornaviruses 113-w

NDS(CF)-74 E 2117
Search for biochemical evidence of virus involvement in chronic neurological diseases 115-w

Infectious Diseases Branch, Summary Report 1-x

NDS(CF)-57 ID 402
Immunology of perinatal infections 15-x

NDS(I)-61 ID 835
Clinical investigations with human volunteers and other populations using viruses, vaccines, and chemotherapeutic agents 21-x

NDS(CF)-72 ID 972
Experimental animal, tissue culture, histopathological and serological investigation of the role of viruses and other micro-organisms in the perinatal period 23-x

NDS(CF)-65 ID 1270
Toxoplasmosis: serological and clinical studies 27-x

NDS(CF)-67 ID 1503
Epidemiologic studies of perinatal infections 29-x

NDS(CF)-69 ID 1731
Isolation of infective agents from chronic diseases 31-x

NDS(CF)-69 ID 1732
Investigation of the role of Mycoplasma spp. in perinatal and neurological diseases 37-x

NDS(CF)-71 ID 1903
Investigation of the etiology and effect of serum and infectious hepatitis in the perinatal period 39-x

NDS(CF)-72 ID 1981
Immunoglobulin M and congenital infections 41-x

NDS(CF)-72 ID 1982
Delayed hypersensitivity in chronic viral diseases 43-x

NDS(CF)-72 ID 1983
Chronic viral infections 45-x

NDS(CF)-72 ID 1984
Maternal infection and pregnancy outcome 49-x

NDS(CF)-72 ID 1985
Isolation and identification of viral antigens and antibody for rapid identification of virus strains and diagnosis of diseases 53-x

NDS(CF)-72 ID 1986
Intrauterine inoculation of fetal monkeys with tissues from patients with chronic diseases and infections 55-x

NDS(CF)-72 ID 1987
Caloric and protein restriction in pregnancy and their effect on measurements and biochemistry of newborn Rhesus monkeys 57-x

NDS(CF)-72 ID 1988
Herpesvirus induction of cervical cancer in Cebus monkeys 59-x

NDS(CF)-72 ID 1989
Transmission of Hepatitis B virus to sub-human primates 61-x

NDS(CF)-72 ID 1990
Chronic lead poisoning in Rhesus monkeys 63-x

NDS(CF)-72 ID 1991
Immunologic studies of congenital infections and chronic infections 65-x

NDS(CF)-72 ID 1992
High risk pregnancies and perinatal infections 69-x

NDS(CF)-72 ID 1993
Amniotic fluid, fetal infection, antibody and infection 71-x

NDS(CF)-72 ID 1994
Clinical studies of chronic infections of the central nervous system 73-x

NDS(CF)-73 ID 2034
Electron microscope immunoperoxidase studies of measles and SSPE viruses 77-x

NDS(CF)-73 ID 2035
Electron microscopic studies of Multiple Sclerosis 81-x

NDS(CF)-73 ID 2036
Immunopathology of chronic neurologic infections 83-x

NDS(CF)-73 ID 2037
Perinatal carcinogenesis in Erythrocebus Patas monkeys 85-x

NDS(CF)-73 ID 2038
Combined clinical viral and immunological investigations of chronic and subacute disorders of the central nervous system 87-x

NDS(CF)-73 ID 2039
Blighted potatoes and birth defects 91-x

NDS(CF)-74 ID 2067
The antenatal diagnosis of anencephaly and spina bifida by maternal serum Alpha Fetoprotein 93-x

NDS(CF)-74 ID 2068
Simian hemorrhagic fever in Patas and Rhesus monkeys 95-x

NDS(CF)-74 ID 2069
Cell mediated immunity and SSPE 99-x

NDS(CF)-74 ID 2070
Affinity chromatography 101-x

NDS(CF)-74 ID 2071
Cerebrospinal fluid electrophoresis- for diagnosis and prognosis of MS, SSPE and Stroke 103-x

Office of Biometry, Summary Report 1-y

Perinatal Research Branch, Summary Report 1-z

NDS(CF)-73 PR/OC 2052
The first year of life 25-z

NDS(CF)-74 PR/OC 2112
Neonatal hyperbilirubinemia 27-z

NDS(CF)-74 PR/PSC 1163
An investigation into the relationship between congenital heart and great vessel anomalies and selected maternal factors as recorded in the Collaborative Perinatal research project 29-z

NDS(CF)-63 PR/PSC 1184
Population dynamics of Tay-Sachs disease and other Sphingolipidoses 31-z

NDS(CF)-65 PR/PSC 1274
Genetic bases of neonatal reflexes 33-z

NDS(CF)-65 PR/PSC 1276
Sequential aspects of occurrence of spontaneous abortion in family histories 35-z

NDS(CF)-66 PR/PSC 1338
The association of mental subnormality with head circumference, congenital malformations, and other conditions of the newborn term infant 37-z

NDS(CF)-67 PR/PSC 1510
The study of maternal effects in the production of congenital malformations 39-z

NDS(CF)-67 PR/PSC 1514
Record linkage of relatives registered in the collaborative study 41-z

NDS(CF)-67 PR/PSC 1515
Rh Hemolytic disease in negro and white infants 43-z

NDS(CF)-67 PR/PSC 1516
Size of placenta in relation to mother-fetus antigenic difference 45-z

NDS(CF)-69 PR/PSC 1748
Neonatal Polycythamia: I. A manifestation of chronic injury during distress 47-z

NDS(CF)-69 PR/PSC 1754
Growth and intellectual development of children from interracial matings 49-z

NDS(CF)-70 PR/PSC 1850
The clinical signs of toxemia of pregnancy and their association with the outcome of pregnancy 51-z

NDS(CF)-70 PR/PSC 1857
The genetics of intellectual and motor performance 53-z

NDS(CF)-71 PR/PSC 1904
The study of labor and delivery 55-z

NDS(CF)-71 PR/PSC 1907
Congenital malformations in the Collaborative Study I. Epidemiologic survey in singletons 59-z

NDS(CF)-71 PR/PSC 1908
A chromosomal study of children in the Collaborative Study 61-z

NDS(CF)-71 PR/PSC 1910
Genetics of obstetric variables and the role of maternal factors in the determination of intelligence and neurological performance 63-z

NDS(CF)-71 PR/PSC 1913
The clinical significance of generalized petechiae at birth 65-z

NDS(CF)-72 PR/PSC 1968
Preschool IQ: Prenatal and early developmental correlates 67-z

NDS(CF)-72 PR/PSC 1969
A study of genetic markers in children of the Collaborative Study 69-z

NDS(CF)-72 PR/PSC 1971
Biosocial factors associated with communicative disorders and competence in children 71-z

NDS(CF)-72 PR/PSC 1972
Relationships between spontaneous speech and other test results at eight years of age 73-z

NDS(CF)-73 PR/PSC 2053
The natural history of congenital toxoplasmosis: Case studies from the Collaborative Perinatal Research Project 75-z

NDS(CF)-73 PR/PSC 2054
Congenital malformations in the Collaborative Study. II. Epidemiology of congenital malformations in multiple births 77-z

NDS(CF)-73 PR/PSC 2055
Congenital malformations in the Collaborative Study. III. Study of the effects of medical, genetic and socioeconomic factors in the occurrence of congenital malformations 79-z

NDS(CF)-73 PR/PSC 2056
Congenital malformations in the Collaborative Study. IV. Maternal diabetes and congenital malformations 81-z

NDS(CF)-73 PR/PSC 2057
Seizures with fever, beginning in the first year of life 83-z

NDS(CF)-73 PR/PSC 2058
Convulsive disorders data analysis group 85-z

NDS(CF)-73 PR/PSC 2059
Cerebral palsy data analysis group 89-z

NDS(CF)-73 PR/PSC 2060
Birthweight-gestational age 93-z

NDS(CF)-72 PR/P 1976
The effects of profound hypothermia in newborn mice: Prolonged circulatory arrest without cerebral damage 127-z

NDS(CF)-73 PR/P 2064
Blood flow study in the intervillous space: Sickling of maternal erythrocytes in the placenta 129-z

NDS(CF)-73 PR/P 2065
Placental transmission of Sickling Erythrocytes from mother to fetus 131-z

NDS(CF)-73 PR/P 2066
The influence of birth rate on neonatal mortality by birth weight, gestational age, race and sex 133-z

NDS(CF)-63 OD/OPE 1144
An instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood 135-z

NDS(CF)-63 OD/OPE 1146
Public Health implications study of perinatal mortality in the Collaborative Study and in the Collaborative Study cities 137-z

Special Programs Branch, Summary Report 1-aa

NDS(CF)-72 SP 2042
Parkinson's Disease and related disorders 19-aa

EXTRAMURAL PROGRAMS

Associate Director's Report 1-bb

Extramural Research 5-bb

NDS(CF)-73 PR/PSC 2061
Physical growth and development task force 95-z

NDS(CF)-73 PR/PSC 2062
Minimal brain dysfunction 97-z

NDS(CF)-74 PR/PSC 2106
Developmental factors associated with mental retardation at age seven 99-z

NDS(CF)-74 PR/PSC 2107
The study of visual abnormalities in the Collaborative Study 103-z

NDS(CF)-74 PR/PSC 2108
Developmental factors associated with learning disorders at age seven 105-z

NDS(CF)-74 PR/PSC 2109
Comprehensive analysis and interpretation of the Collaborative Perinatal Project data on congenital malformations 109-z

NDS(CF)-74 PR/PSC 2110
Delayed motor development at one year: Antecedents and intellectual and neurological outcomes 113-z

NDS(CF)-74 PR/PSC 2111
A Centennial Bibliography of Huntington's Chorea 115-z

NDS(CF)-66 PR/P 1345
Influencing factors in Sudden Unexpected Death 117-z

NDS(CF)-70 PR/P 1859
Mental and motor development in Monozygotic Co-Twins with dissimilar birthweights 119-z

NDS(CF)-72 PR/P 1973
Renal glomerular development in infants of diabetic mothers 121-z

NDS(CF)-72 PR/P 1974
The significance of Meconium staining in neonatal deaths 123-z

NDS(CF)-72 PR/P 1975
Organ weight change of infants with pulmonary hyaline membranes 125-z

NDS(CF)-73 PR/PSC 2061
Physical growth and development task force 95-z

NDS(CF)-73 PR/PSC 2062
Minimal brain dysfunction 97-z

NDS(CF)-74 PR/PSC 2106
Developmental factors associated with mental retardation at age seven 99-z

NDS(CF)-74 PR/PSC 2107
The study of visual abnormalities in the Collaborative Study 103-z

NDS(CF)-74 PR/PSC 2108
Developmental factors associated with learning disorders at age seven 105-z

NDS(CF)-74 PR/PSC 2109
Comprehensive analysis and interpretation of the Collaborative Perinatal Project data on congenital malformations 109-z

NDS(CF)-74 PR/PSC 2110
Delayed motor development at one year: Antecedents and intellectual and neurological outcomes 113-z

NDS(CF)-74 PR/PSC 2111
A Centennial Bibliography of Huntington's Chorea 115-z

NDS(CF)-66 PR/P 1345
Influencing factors in Sudden Unexpected Death 117-z

NDS(CF)-70 PR/P 1859
Mental and motor development in Monozygotic Co-Twins with dissimilar birthweights 119-z

NDS(CF)-72 PR/P 1973
Renal glomerular development in infants of diabetic mothers 121-z

NDS(CF)-72 PR/P 1974
The significance of Meconium staining in neonatal deaths 123-z

NDS(CF)-72 PR/P 1975
Organ weight change of infants with pulmonary hyaline membranes 125-z

NDS(CF)-72 PR/P 1976
The effects of profound hypothermia in newborn mice: Prolonged circulatory arrest without cerebral damage 127-z

NDS(CF)-73 PR/P 2064
Blood flow study in the intervillous space: Sickling of maternal erythrocytes in the placenta 129-z

NDS(CF)-73 PR/P 2065
Placental transmission of Sickling Erythrocytes from mother to fetus 131-z

NDS(CF)-73 PR/P 2066
The influence of birth rate on neonatal mortality by birth weight, gestational age, race and sex 133-z

NDS(CF)-63 OD/OPE 1144
An instrument for the conduct of a retrospective study of seizures, cerebral palsy, mental retardation and other neurological and sensory disorders of infancy and childhood 135-z

NDS(CF)-63 OD/OPE 1146
Public Health implications study of perinatal mortality in the Collaborative Study and in the Collaborative Study cities 137-z

Special Programs Branch, Summary Report 1-aa

NDS(CF)-72 SP 2042
Parkinson's Disease and related disorders 19-aa

EXTRAMURAL PROGRAMS

Associate Director's Report 1-bb

Extramural Research 5-bb

PROJECTS LISTED NUMERICALLY

PROJECT NUMBER	PAGE	PROJECT NUMBER	PAGE
NDS(I)-54 SN/OC 100(c)	7-g	NDS(I)-63 DMN/CIT 1026(c)	17-j
NDS(I)-55 SN/OC 200(c)	13-g	NDS(I)-63 MN/OC 1034(c)	21-f
NDS(CF)-55 E 201	19-w	NDS(I)-63 MN/OC 1037(c)	27-f
NDS(I)-56 SN/OC 304(c)	15-g	NDS(I)-63 MN/OC 1039(c)	31-f
NDS(CF)-57 ID 402	15-x	NDS(I)-63 SN/NR 1047(c)	29-g
NDS(I)-60 DMNB/ND 706(c)	5-j	NDS(I)-63 MN/AP 1049(c)	73-f
NDS(I)-61 LNC/AAC 810	5-p	NDS(I)-63 MN/AP 1050(c)	75-f
NDS(I)-61 LNC/EC 811	7-p	NDS(I)-63 LNNS/NDR 1054	21-k
NDS(I)-61 LNC/EC 813	13-p	NDS(I)-63 LNNS/ENP 1066	49-k
NDS(I)-61 DMN/EG 815	11-j	NDS(CF)-63 E 1103	25-w
NDS(I)-61 DMN/EG 816	15-j	NDS(CF)-63 OD/OPE 1144	135-z
NDS(CF)-61 ID 835	21-x	NDS(CF)-63 OD/OPE 1146	137-z
NDS(I)-62 SN/OC 907(c)	19-g	NDS(CF)-63 PR/PSC 1163	29-z
NDS(I)-62 SN/OC 913(c)	23-g	NDS(CF)-63 PR/PSG 1184	31-z
NDS(I)-62 MN/OC 915(c)	9-f	NDS(I)-65 MN/OC 1189(c)	41-f
NDS(I)-62 MN/OC 917(c)	15-f	NDS(I)-65 MN/OC 1190(c)	43-f
NDS(I)-62 MN/AP 926(c)	71-f	NDS(I)-65 MN/OC 1191(c)	49-f
NDS(I)-62 LB/OC 935	7-n	NDS(I)-65 MN/OC 1192(c)	53-f
NDS(I)-62 LB/OC 936	9-n	NDS(I)-65 MN/OC 1193(c)	59-f
NDS(I)-62 LB/OC 939	11-n	NDS(I)-65 SN/NR 1195(c)	33-g
NDS(I)-62 LB/OC 940	13-n	NDS(I)-65 LMB/RN 1208	23-r
NDS(CF)-65 CNNS 969	11-h	NDS(I)-65 LNNS/FN 1231	63-k
NDS(CF)-62 ID 972	23-x	NDS(I)-65 LNP/SP 1239	3-m
NDS(I)-63 SN/OC 1025(c)	25-g	NDS(I)-65 LB/OC 1240	15-n

PROJECT NUMBER	PAGE
NDS(I)-65 LMB/DB 1244	7-r
NDS(I)-66 SN/OC 1245(c)	39-g
NDS(CF)-65 ID 1270	27-x
NDS(CF)-65 PR/PSC 1274	33-z
NDS(CF)-65 PR/PSC 1276	35-z
NDS(CF)-65 CNNS 1282	39-h
NDS(I)-66 LNNS/NDR 1303	23-k
NDS(I)-66 DMN/EG 1309	21-j
NDS(CF)-66 E 1319	27-w
NDS(CF)-66 PR/PSC 1338	37-z
NDS(CF)-66 PR/P 1345	117-z
NDS(I)-66 LPP 1388	7-q
NDS(I)-67 SN/NR 1413(c)	41-g
NDS(I)-67 MN/AP 1416(c)	77-f
NDS(I)-67 SN/OC 1417(c)	45-g
NDS(I)-67 SN/OC 1424(c)	47-g
NDS(I)-67 LNNS/FN 1442	67-k
NDS(I)-67 LNNS/NC 1443	95-k
NDS(I)-67 LNNS/ENP 1449	51-k
NDS(I)-67 DMN/NM 1457	25-j
NDS(I)-67 ODIR 1460	1-e
NDS(I)-67 LPP 1464	9-q
NDS(I)-67 LNC/PM 1480	33-p
NDS(I)-67 LNC/PM 1481	37-p
NDS(CF)-67 E 1487	29-w
NDS(CF)-67 E 1488	33-w
NDS(CF)-67 E 1490	37-w
NDS(CF)-67 E 1496	39-w
NDS(CF)-67 ID 1503	29-x
NDS(CF)-67 PR/PSC 1510	39-z
NDS(CF)-67 PR/PSC 1514	41-z
NDS(CF)-67 PR/PSC 1515	43-z
NDS(CF)-67 PR/PSC 1516	45-z
NDS(I)-68 DMN/CIT 1523(c)	27-j
NDS(I)-68 SN/OC 1526(c)	49-g
NDS(I)-68 SN/OC 1527(c)	51-g
NDS(I)-68 ODIR 1554	3-e
NDS(I)-68 LNNS/NDR 1586	27-k
NDS(I)-68 LNNS/NC 1587	97-k
NDS(CF)-68 E 1594	41-w
NDS(CF)-68 E 1597	43-w
NDS(CF)-68 E 1598	45-w
NDS(CF)-68 E 1605	47-w
NDS(I)-69 SN/NR 1654	53-g
NDS(I)-69 DMN/CIT 1655(c)	31-j
NDS(I)-69 SN/OC 1658(c)	55-g
NDS(I)-69 LNP/CB 1659(c)	13-m
NDS(I)-69 LNNS/ENP 1676	53-k
NDS(I)-69 LNNS/NC 1678	99-k
NDS(I)-69 LNNS/FN 1684	71-k
NDS(I)-69 LNLC/OC 1686	7-l
NDS(I)-69 LNLC/OC 1687	13-l

PROJECT NUMBER	PAGE
NDS(I)-69 LNLC/OC 1688	17-1
NDS(I)-69 LNP/SP 1690	7-m
NDS(I)-69 LEN/OC 1693	7-o
NDS(I)-69 LEN/OC 1694	11-o
NDS(I)-69 LPP 1699	13-q
NDS(I)-69 MN/OC 1728	63-f
NDS(CF)-69 ID 1731	31-x
NDS(CF)-69 ID 1732	37-x
NDS(CF)-69 PR/PSC 1748	47-z
NDS(CF)-69 PR/PSC 1754	49-z
NDS(CF)-69 PR/P 1772	91-z
NDS(CF)-69 E 1774	49-w
NDS(CF)-69 E 1777	51-w
NDS(CF)-69 E 1779	53-w
NDS(I)-70 ODIR 1789	5-e
NDS(I)-70 SN/NR 1791(c)	59-g
NDS(I)-70 MN/OC 1792(c)	67-f
NDS(I)-70 LNNS/CN 1802	21-k
NDS(I)-70 LNNS/CN 1803	23-k
NDS(I)-70 LNNS/NC 1805	101-k
NDS(I) DMN/EG 1808	35-j
NDS(I)-70 MN/AP 1809(c)	79-f
NDS(CF)-70 E 1832	55-w
NDS(CF)-70 E 1833	57-w
NDS(CF)-70 E 1835	59-w
NDS(CF)-70 E 1836	61-w
NDS(CF)-70 E 1837	63-w
NDS(CF)-70 E 1838	65-w
NDS(CF)-70 E 1839	67-w
NDS(CF)-70 E 1843	69-w
NDS(CF)-70 E 1844	71-w
NDS(CF)-70 E 1845	73-w
NDS(CF)-70 PR/PSC 1850	51-z
NDS(CF)-70 PR/PSC 1857	53-z
NDS(CF)-70 PR/P 1859	119-z
NDS(I)-71 SN/NR 1866	61-g
NDS(I)-71 LNNS/NC 1879	77-k
NDS(I)-71 LNNS/NC 1880	79-k
NDS(I)-71 LNNS/FN 1881	73-k
NDS(I)-71 LNNS/CNC 1884	7-k
NDS(I)-71 LNNS/CNC 1885	9-k
NDS(I)-71 LMB/DB 1886	11-r
NDS(I)-71 LNP/GP 1889	17-m
NDS(I)-71 LNP/NI 1892	39-m
NDS(I)-71 LNP/NI 1895	41-m
NDS(I)-71 LEN/OC 1897	15-o
NDS(CF)-71 ID 1903	39-x
NDS(CF)-71 PR/PSC 1904	55-z
NDS(CF)-71 PR/PSC 1907	59-z
NDS(CF)-71 PR/PSC 1908	61-z

PROJECT NUMBER	PAGE	PROJECT NUMBER	PAGE
NDS(CF)-71 PR/PSC 1910	63-z	NDS(I)-72 LMB/DB 1962	15-r
NDS(CF)-71 PR/PSC 1913	65-z	NDS(I)-72 LMB/DB 1963	19-r
NDS(CF)-71 E 1917	75-w	NDS(I)-72 LNNS/NC 1965	103-k
NDS(CF)-71 E 1920	77-w	NDS(I)-72 LB/OC 1966	25-n
NDS(CF)-71 E 1921	79-w	NDS(I)-72 LB/OC 1967	27-n
NDS(CF)-71 E 1922	81-w	NDS(CF)-72 PR/PSC 1968	67-z
NDS(CF)-71 E 1923	83-w	NDS(CF)-72 PR/PSC 1969	69-z
NDS(CF)-71 E 1924	85-w	NDS(CF)-72 PR/PSC 1971	71-z
NDS(CF)-71 E 1925	89-w	NDS(CF)-72 PR/PSC 1972	73-z
NDS(CF)-71 E 1926	91-w	NDS(CF)-72 PR/P 1973	121-z
NDS(CF)-71 E 1927	93-w	NDS(CF)-72 PR/P 1974	123-z
NDS(CF)-71 E 1928	95-w	NDS(CF)-72 PR/P 1975	125-z
NDS(CF)-71 E 1930	97-w	NDS(CF)-72 PR/P 1976	127-z
NDS(CF)-71 AN 1932	31-v	NDS(CF)-72 E 1977	99-w
NDS(CF)-71 AN 1933	33-v	NDS(CF)-72 E 1980	101-w
NDS(I)-72 EEG/CN 1939a	7-i	NDS(CF)-72 ID 1981	41-x
NDS(I)-72 EEG/CN 1939b	9-i	NDS(CF)-72 ID 1982	43-x
NDS(I)-72 EEG/CN 1940(c)	11-i	NDS(CF)-72 ID 1983	45-x
NDS(I)-72 LNNS/CNC 1942	13-k	NDS(CF)-72 ID 1984	49-x
NDS(I)-72 LNP/GP 1943	21-m	NDS(CF)-72 ID 1985	53-x
NDS(I)-72 LNP/GP 1944	25-m	NDS(CF)-72 ID 1986	55-x
NDS(I)-72 LNP/GP 1945	29-m	NDS(CF)-72 ID 1987	57-x
NDS(I)-72 LB/OC 1947	17-n	NDS(CF)-72 ID 1988	59-x
NDS(I)-72 LB/OC 1948	19-n	NDS(CF)-72 ID 1989	61-x
NDS(I)-72 LB/OC 1950	21-n	NDS(CF)-72 ID 1990	63-x
NDS(I)-72 LEN/OC 1952	19-o	NDS(CF)-72 ID 1991	65-x

PROJECT NUMBER	PAGE	PROJECT NUMBER	PAGE
NDS(CF)-72 ID 1992	69-x	NDS(I)-73 LNP/SP 2019	9-m
NDS(CF)-72 ID 1993	71-x	NDS(I)-73 LPP 2020	15-q
NDS(CF)-72 ID 1994	73-x	NDS(I)-73 LPP 2021	19-q
NDS(I)-73 LNNS/CN 1995	25-k	NDS(I)-73 LPP 2022	21-q
NDS(I)-73 LNNS/CN 1996	29-k	NDS(I)-73 DMN/CIT 2024	41-j
NDS(I)-73 LNNS/CP 1997	33-k	NDS(I)-73 LMB/MV 2026	27-r
NDS(I)-73 LNNS/CP 1998	35-k	NDS(CF)-73 ID 2034	77-x
NDS(I)-73 LNNS/NC 1999	81-k	NDS(CF)-73 ID 2035	81-x
NDS(I)-73 LNNS/NC 2000	85-k	NDS(CF)-73 ID 2036	83-x
NDS(I)-73 LNNS/NC 2001	87-k	NDS(CF)-73 ID 2037	85-x
NDS(I)-73 LNNS/ENP 2002	55-k	NDS(CF)-73 ID 2038	87-x
NDS(I)-73 LNNS/ENP 2003	57-k	NDS(CF)-73 ID 2039	91-x
NDS(I)-73 LNNS/ENP 2004	59-k	NDS(CF)-73 SP 2042	1g-aa
NDS(I)-73 LNNS/ENP 2005	61-k	NDS(CF)-73 E 2045	103-w
NDS(I)-73 LNNS/CNC 2006	17-k	NDS(CF)-73 E 2046	105-w
NDS(I)-73 LNNS/CNC 2007	19-k	NDS(CF)-73 BEI 2047	13-u
NDS(I)-73 SN/NR 2008	65-g	NDS(CF)-73 AN 2051	45-v
NDS(I)-73 SN/OC 2009	69-g	NDS(CF)-73 PR/OC 2052	25-z
NDS(I)-73 SN/OC 2010	71-g	NDS(CF)-73 PR/PSC 2053	75-z
NDS(I)-73 EEG/CN 2011	15-i	NDS(CF)-73 PR/PSC 2054	77-z
NDS(I)-73 EEG/CN 2012	17-i	NDS(CF)-73 PR/PSC 2055	79-z
NDS(I)-73 EEG/CN 2013	19-i	NDS(CF)-73 PR/PSC 2056	81-z
NDS(I)-73 EEG/CN 2014(c)	21-i	NDS(CF)-73 PR/PSC 2057	83-z
NDS(I)-73 LNLC/OC 2015	21-l	NDS(CF)-73 PR/PSC 2058	85-z
NDS(I)-73 LNP/CB 2016	15-m	NDS(CF)-73 PR/PSC 2059	89-z
NDS(I)-73 LNP/GP 2017	33-m	NDS(CF)-73 PR/PSC 2060	93-z
NDS(I)-73 LNP/GP 2018	37-m	NDS(CF)-73 PR/PSC 2061	95-z

PROJECT NUMBER	PAGE	PROJECT NUMBER	PAGE
NDS(CF)-73 PR/PSC 2062	97-z	NDS(I)-74 LB/OC 2089	37-n
NDS(CF)-73 PR/P 2064	129-z	NDS(I)-74 LB/OC 2090	39-n
NDS(CF)-73 PR/P 2065	131-z	NDS(I)-74 LB/OC 2091	43-n
NDS(CF)-73 PR/P 2066	133-z	NDS(I)-74 LB/OC 2092	49-n
NDS(CF)-74 ID 2067	93-x	NDS(I)-74 LNNS/CP 2093	39-k
NDS(CF)-74 ID 2068	95-x	NDS(I)-74 EEG/CN 2094	23-i
NDS(CF)-74 ID 2069	99-x	NDS(I)-74 EEG/CN 2095	25-i
NDS(CF)-74 ID 2070	101-x	NDS(I)-73 EEG/CN 2096	27-i
NDS(CF)-74 ID 2071	103-x	NDS(CF)-74 AN 2097	35-v
NDS(I)-74 SN/NR 2072(c)	73-g	NDS(CF)-74 AN 2098	37-v
NDS(I)-74 SN/NR 2073(c)	75-g	NDS(CF)-74 AN 2099	39-v
NDS(I)-74 LNC/NDR 2074	31-p	NDS(CF)-74 AN 2100	41-v
NDS(I)-74 LNC/EC 2075	19-p	NDS(CF)-74 AN 2101	43-v
NDS(I)-74 LNC/AAC 2076	9-p	NDS(CF)-74 CD 2102	13-t
NDS(I)-74 LNC/AAC 2077	11-p	NDS(I)-74 DMN/CIT 2103	45-j
NDS(I)-74 LNLC/OC 2078	25-l	NDS(I)-74 LNNS/CP 2104	41-k
NDS(I)-74 LNLC/OC 2079	27-l	NDS(I)-74 LNNS/CP 2105	45-k
NDS(I)-74 LNLC/OC 2080	31-l	NDS(CF)-74 PR/PSC 2106	99-z
NDS(I)-74 LPP 2081	23-q	NDS(CF)-74 PR/PSC 2107	103-z
NDS(I)-74 LNNS/CN 2082	31-k	NDS(CF)-74 PR/PSC 2108	105-z
NDS(I)-74 LNNS/NC 2083	89-k	NDS(CF)-74 PR/PSC 2109	109-z
NDS(I)-74 LNNS/NC 2084	91-k	NDS(CF)-74 PR/PSC 2110	113-z
NDS(I)-74 LNNS/NC 2085	93-k	NDS(CF)-74 PR/PSC 2111	115-z
NDS(I)-74 LNNS/NC 2086	105-k	NDS(CF)-74 PR/OC 2112	27-z
NDS(I)-74 LB/OC 2087	29-n	NDS(CF)-74 E 2113	107-w
NDS(I)-74 LB/OC 2088	33-n	NDS(CF)-74 E 2114	109-w

PROJECT NUMBER	PAGE	PROJECT NUMBER	PAGE
NDS(CF)-74 E 2115	111-w		
NDS(CF)-74 E 2116	113-w		
NDS(CF)-74 E 2117	115-w		
NDS(CF)-74 AN 2118	47-v		
NDS(CF)-74 AN 2119	49-v		
NDS(CF)-74 AN 2120	51-v		

ANNUAL REPORT
July 1, 1973 through June 30, 1974
National Institute of Neurological Diseases and Stroke
National Institutes of Health

Director's Report

Fiscal Year 1974 has been a turbulent year, a year of restlessness and ferment. It has been characterized by a complete change of the top staffing of the NIH, by changes in the directors of four institutes plus the creation of the first new institute in five years, and by changes in the scientific directors of five institutes. The fiscal situation and the status of training programs have frequently bordered on the chaotic. On the balance, however, FY 1974 proved to be a considerably better year for the NINDS than the preceding year. Downward trends in budget and personnel were reversed, and the stagnation of interregunums at both the director's and the scientific director's levels has given way to new leadership in these two key positions.

The mission and programs of the NINDS have gained greater attention and recognition at the NIH and DHEW levels as a result of a number of substantive contacts with Drs. Stone and Edwards and Secretary Weinberger. The importance of the Institute's concern with research in the communicative disorders has received renewed emphasis by new initiatives in our on-campus programs. And attention is being focussed on newly emerging frontiers in neuropharmacology and neuroimmunology. This is not to say that there have not been problems and challenges. The perception of the needs to exercise fiscal contraints and program restructuring in the NINDS information network centers met with strongly mixed reactions and provocative responses. The request by the Congress for study and support of chiropractic research has tested our ingenuity to deal responsibly with the realities of research issues (by planning for FY 1975 a workshop on the research status of spinal manipulative therapy while avoiding the morass of the health care delivery controversy. The National Advisory Commission on Multiple Sclerosis completed its work and submitted a detailed report with carefully formulated and challenging recommendations. Public response has at times taxed our respondent resources, and apparently we may expect a sequel in the proposed epilepsy commission. An NANDS Council Subcommittee is in the midst of evaluating the current status and future potential of central nervous system regeneration. Within the NINDS the needs for reorganizing our ways of conducting extramural business, for infusing more flexibility into on-campus research programs, and for developing more effective planning and evaluation capabilities are occupying much of our attention. These, then, are some of the facets of the rather extraordinary collage that has been FY 1974.

In the NINDS extramural programs (EP), an increased appropriation, plus release of the so-called impounded FY 1973 funds (amounting to some $23 million), permitted a more realistic funding of meritorious research grants. Thus, instead of the originally forecasted funding of less than

20 percent of approved grants, the Institute has been able to fund over 40 percent of approved grants, including a much healthier proportion of new and renewal grant applications. At the close of FY 1974, a total of 1065 research grants was in effect, representing an investment of almost $70 million. The fiscal problem of coping with the release late in FY 1974 of FY 1973 impounded funds was met by resorting to two-year grants in many cases, in order to provide a reasonable award period to the investigators without dislocating the FY 1975 commitment base. To acknowledge that this traditional NINDS managerial practice created departmental and Congressional controversy is to understate the case. Nevertheless, we believe that judicious use of multi-year funding is an invaluable managerial and fiscal tool, and we urge that institutes be authorized to use a modest percentage (e.g., 6 percent) of extramural funds in this way. The brighter research grant picture must be tempered by the growing problem of rising costs. Over the period 1962-1974, the NINDS appropriation available for research grants have nearly doubled (from $36 million to $70 million) but the average cost per individual research grant has increased nearly 2.5-fold (from $22,000 to $49,900). By contrast, the average cost per program project or center grant has remained virtually constant (at $250 - 275,000) over the 12-year period, although the percentage of the total research grant budget devoted to these targeted categories has increased from 10 to 31 percent. A major consequence of these trends is a decrease in the number of individual research grants of nearly 40 percent over the 12-year period.

The training situation is about as confusing as it possibly could be. The reversal of the FY 1973 phase-out; the release of FY 1973 impounded funds; the initiation of the "new" institutional fellowship (Weinberger) program; and finally the Congressional abolition of all these programs and substitution of a new set of programs based on demonstrated manpower needs and embellished with pay-back provisions--all have both the biomedical community and the Bethesda staffs thoroughly confused. The NINDS had already embarked upon manpower surveys in the five major areas of its responsiblity, with reports from all but one contractor due early in FY 1975. Hopefully these can provide the basis for interim training support until the newly-required NAS data become available. We must avoid any significant hiatus in training research and teacher personnel in critical areas. Certainly the impact of so many changes in direction and continuity can be devastating at a time when it is essential to maintain training momentum in many areas of the neurosciences.

At the conclusion of FY 1974, the NINDS was funding research grants along a wide front of programs. A total of 89 program project and center grants were in force, including: 4 clinical research centers in Parkinsonism and disorders of aging; 14 stroke clinical research centers and 11 stroke acute care research units; 4 head injury clinical research centers; 5 acute spinal cord injury clinical research centers; 6 epilepsy clinical research centers; 3 neuromuscular disorder clinical research centers; 4 multiple sclerosis clinical research centers; and 2 clinical research centers and 2 outpatient research units in the communicative disorders. Among individual research grants funded during FY 1974 investigations currently in progress include:

studies at the molecular level on excitatory and inhibitory receptors; functional effectiveness of muscle reinnervation with autonomic nerves; continuing biochemical studies on catecholamine (dopamine) metabolism in Parkinsonism and Huntington's disease and trials of newer pharmacological approaches to therapy; evidence for receptor-mediated feedback control of dopamine synthesis; probing for masked or latent viruses in human and simian brain tissue explants; immunological studies on SSPE and biochemical and genetic studies on mutant measles viruses; evaluation of various viral models of demyelination for relevance to multiple sclerosis; application of latest techniques for isolation of viral and host DNA and for hybridization or annealing to test for identical genetic sequences; studies on the clinicopathological significance of herpes virus in the CNS; use of visna virus as a model for slow virus-induced demyelinating diseases, including immunological and nucleic acid studies; new in vitro tests for evaluating drugs for myasthenia gravis; potentialities for identifying the majority of female carriers of the Duchenne type of muscular dystrophy; identification of glial cells as the normal physiological source for nerve growth factor in the CNS; concentration on mechanisms of autoregulation of local cerebral or cord blood flow in stroke and head or cord injury and their correlation with local transmitter and biogenic amines; surveys on factors responsible for regional differences in stroke morbidity and mortality; evaluations of newer non-invasive techniques for monitoring stroke, head injury and tumor patients; role of increased external potassium in seizures and in ischemia associated with stroke or trauma; study of the "kindling" phenomenon in epileptic foci; elucidation of normal factors and pathological variations in cellular immunological phenomena; further details on the mechanisms by which sound vibrations are transduced into nerve impulses by cochlear hair cells; in depth studies on secretory otitis media; basic information requisite for design of multichannel cochlear implants; comparative studies on hearing; clinicopathological correlations in laryngeal cancer; behavioral and biofeedback techniques in research on stuttering.

Although the NINDS collaborative and field research (C&FR) contract programs remain at about 10 percent of the expenditures in the research grant program, the combined contract and C&FR on-campus studies represent a significant part of the overall Institute research mission. During FY 1974 the NINDS funded 116 contracts, in comparison to 87 in the previous year. The creation of a research contract office and the recruitment of Dr. Mathilde Solowey to head its technical merit review unit represented major improvements in the management of our contract program as recommended by the Cooper report. A C&FR Section on Communicative Disorders was established, and Dr. Lois Elliott, an expert on speech and hearing disorders, was recruited as its head. With the impending departure of the chief of the C&FR Epidemiology Branch, much of the branch's activities (including the Guam facility) have been transferred to the office of the Director of Intramural Research, and the remainder will be housed in the C&FR Office of Biometry as the latter unit moves into a number of incidence and prevalence surveys. The NINDS continues to manage 30 projects worth $900,000 in the PL-480 (counterpart funds) program, with projects in Egypt, India, Israel, Poland and Yugoslavia. Of the 30 projects, 10 have NINDS scientists as project officers. Indications are

that most of these programs will shortly phase out, as counterpart funds are used up or are cancelled. Hopefully, alternatives, such as bilateral agreements, can be developed, since the collaboration with foreign scientists in certain unique projects has been most effective.

Among the many findings and developments in the various C&FR programs the following are representative: completion of the data collection phase in the perinatal collaborative study; correlation of high measles antibody titre in CSF with the diagnosis of SSPE; trials of interferon in SSPE; indication of a serum factor blocking the immune lymphocytes from attacking SSPE-infected cells; implementation of the contract for an epidemiological survey of multiple sclerosis in the Shetland and Orkney Islands, where the incidence is the highest in the world; evidence in Chamorro brains from Guam of oncornavirus-like genetic material, a possible lead in the ALS and PD puzzle; approval of the first new anticonvulsant drug (carbamazepine) in 14 years, plus promising data on several other new anticonvulsants; initiation at 11 centers of the feasibility stage of the comprehensive epilepsy program; establishment of a coordinated approach by NINDS, NIEHS and NIOSH of research on problems of noise pollution.

Several important changes have taken place in the NINDS intramural research (IR) program. During FY 1974 a search committee was convened to recommend a successor for Dr. Henry Wagner, who requested in June 1972 to be relieved as Director of Intramural Research in order to return to laboratory research. As a result of the search committee's diligent efforts we have been able to recruit an outstanding new Scientific Director in Dr. Thomas N. Chase, with expertise in clinical neurology and neuropharmacology. He is expected to enter on duty at the beginning of FY 1975 and will also head the new Laboratory of Neuropharmacology, which will provide the NINDS intramural program for the first time with both experimental animal and clinical research capabilities in disorders of transmitter and biogenic amine metabolism. Early in FY 1974 the Laboratory of Neuro-otolaryngology was created, with Dr. Jorgen Fex, an internationally recognized expert in neurophysiology of auditory mechanisms, recruited as its chief. This laboratory, initially proposed 20 years ago, has now become a reality as the first intramural research program devoted to communicative disorders. As such it should provide a focus for much needed research training in this field. Plans have been completed for creation of anothor new research program, the Laboratory of Neuro-immunology, with recruitement of an outstanding clinical neurologist and immunologist, Dr. Dale McFarlin, as its chief. This laboratory will address the growing need for immunological capabilities in many intramural projects. notably in research on slow viruses and diseases of cellular immunity like SSPE and multiple sclerosis. Neuro-immunology is a very new and sparsely represented research specialty, so that a major responsiblity of the new IR laboratory will be training additional investigators for this critically important field. At the request of the Department of Commerce the unit of the Laboratory of Central Nervous System Studies at Patuxent was vacated and transferred in part to the Bldg. 36 laboratories, partly to primate holding facilities at Gulf South Research Institute in New Iberia, La.,

and partly to temporary space at the Frederick Cancer Research Center, pending completion of renovations of the NINDS building there. A consequence of the various foregoing development has been the necessity to move all non-laboratory activities in Bldg. 36 out to office-type space. Thus, the C&FR offices, contracts office, and administrative components of the C&FR Applied Neurology Branch are being or have been displaced by IR laboratory programs. Space is still at a real premium and further solutions for increasing flexibility for new or changing laboratory operations are being developed.

Stimulating and important findings or developments continue to emerge from IR research projects. Representatives are the following: use of the vesicular stomatitis virus as a model for studies of the control of RNA-virus replication with special attention to defective viral particles; immunosuppression of the rejection of homografted ganglia by means of tolerance induction in the host and consequent survival of neurons with regeneration of processes and establishment of functional connections; correlation of major changes in membrane lipids (increased unsaturation) with changes in Na-K-ATPase activity during hibernation; studies of cerebral ischemia and the no-reflow phenomenon in the gerbil stroke model with correlation of ischemia with cytotoxic edema; identification of increased serotonin from platelets as the crucial factor in vasogenic edema following cold injury; use of freeze fracture electron microscopy to delineate distinctive features of synapses; detailing by electron microscopy of the sequence of events in discharge of synaptic vesicles and resorption and recycling of vesicle membrane (in close analogy to exocytosis of membrane-enclosed viral particles); development of several new types of electrodes that permit for the first time chronic recording respectively from dorsal root-ganglia, from nerves, and from muscle motor units in unanesthetized animals; several fruitful contracts in the neural prosthesis program; additional evidence for transmission of Creutzfeldt-Jakob disease (in one case via a corneal transplant; in others among Libyan Jews, by consumption of sheep brains; studies under contract to probe human brain tissue for reverse transcriptase (RNA viruses) and by viral DNA hybridization in view of the extensive range of viruses recoverable from non-human primate brains; successful purification of the scrapie agent to a preparation behaving more like a conventional virus; prolonged effectiveness of enzyme replacement therapy in Gaucher's disease; identification of a patient with an inherited defect in the synthesis of gangliosides more complex than hematoside--the first example of an anabolic defect in the group of lipid storage diseases; similarities in the blocks of ganglioside synthesis by virally-transformed cells and by cells treated with chemical carcinogens or X-irradiation; evidence that the glycoprotein characteristic of myelin may be essential for myelinogenesis as a result of studies on myelination in normal animals and in the Quaking and Jimpy mutants; initiation of experimental animal and clinical pilot studies of cerebellar electrode implants and stimulation for control of seizures.

During FY 1974 these and other IR studies were carried out by 110 doctoral staff scientists and 33 visiting scientists and guest workers plus sup-

porting staff on 131 projects, with publication of 211 full papers reporting on their research. Clinical research activities involved 513 inpatient admissions and 11,078 patient days, 1441 outpatient visits, 410 neurological consultations for other institutes, 400 muscle biopsies, 125 neurosurgical procedures, and 1300 EEG examinations. There were two special developments. Arrangements were made for the Yakovlev collection of serially sectioned whole mounts of human brains to be transferred from inadequate storage in Boston to permanent housing at the AFIP. Transfer, curator and maintenance costs are being covered by contracts funded by NINDS with an IR scientist as project officer. After years of inconclusive negotiations this unique anatomical and pathological collection is now properly preserved and accessible for study by any qualified scientist. Similarly, negotiations are far advanced to transfer anthropological data and materials with their curator from the IR-LCNSS to the Smithsonian Institution. This latter material has been acquired as the LCNSS scientists study immunologically "virgin" primitive peoples who represent unequalled "sentinel populations" for revealing the focal microbial agents that infect man in his environment.

Special concern continues for equal employment opportunity (EEO) programs in the NINDS. An obviously dedicated staff, including coordinator, counsellor and committee members from each administrative unit of the Institute, has been the key to effective implementation of EEO activities and to responsive dealing with the many day-to-day employee-management problems. The executive staff of this Institute is keenly aware of the effectiveness of this managerial partnership. Highlights during FY 1974 have been: issuance of a comprehensive EEO report to all NINDS employees; increased staffing of minority and women employees in higher grades; initiation of EEO seminars for NINDS supervisory personnel; active participation of EEO staff in search committees and key recruitment actions; revision and updating of the NINDS Affirmative Action Plan; and continuation of the minority teaching and fellowship programs in the neurosciences. This last program contracted for by NINDS with several marine biological laboratories primarily for summer work by faculty from minority colleges has been especially valuable. It is to be hoped that the current confusion over training and fellowship programs will not inadvertently jeopardize this modest but highly desirable program. Particular efforts are still needed to ensure placement of more minority and women scientists and administrators in higher grade positions.

Last year this report concluded with a brief summary of the dimensions and importance of the research missions of the NINDS. Various studies by others during FY 1974 indicate that the dimensions may be even more formidable than our admittedly imperfect statistics suggest. Rather than 2 million epileptics, there may be 4 million in the U.S. Their cost to the Nation may be well over $4 billion rather than the $2 billion usually cited. There may be four or five-fold the number of Huntington's disease patients previously estimated. Incidence and prevalence surveys are now being undertaken by the NINDS to provide better bases for ascertaining the magnitude of such problems. In other words the challenge to the NINDS is becoming significantly greater. This is particularly true in the

genetic and viral disorders of the nervous system. Unless proved to the contrary, we must assume that the human central nervous system may harbor a great many slow and latent viruses, each of which is a potential source for demyelination, dementia, or neoplasia. A clear perception of the precise dimensions of this problem is needed now. For major problems like stroke and CNS trauma, more major advances in prevention, monitoring and therapy are needed. The obviously burgeoning interest in neurobiology and the neurosciences may presage the development of that critical mass needed to make significant impacts on such major research challenges. This will not happen unless, over the next decade, continuities of funding of research and infusion of trained personnel can be adequately assured.

Office of Scientific and Health Reports

NINDS information and publications activities are centered within the Office of Scientific and Health Reports, formerly the Office of Public Affairs. In consonance with its new title and function, the Office of Scientific and Health Reports includes three sections: Scientific Publications, Health Reports, and Public Inquiries.

The Office is responsible for advising the Director and his executive staff, on the effective interpretation and utilization of Institute conducted and supported research findings for many audiences. These include Congress, the Department and other areas of Government, scientists, physicians, voluntary agencies, and directly and indirectly the general public. The program is one both of initiating and implementing broad projects and responding to Congressional, Departmental, Institute, and public requests.

The three sections of the office not only produce the scientific publications, health reports, and answers to public inquiries, but also serve as an information resource on the many disease categories for which the Institute is responsible.

Public Inquiries Section

Staff members of this section spend most of their time responding to letters and phone inquiries. In the past year, more than 3,000 inquiries required individually written letters answering specific questions. For many of these, research was necessary. For many, it was important to coordinate information with intramural scientists and occasionally with Institute grantees.

Congressional mail was especially heavy last year as a result of continuing budget changes. Publication of the recommendations of the National Advisory Multiple Sclerosis Commission resulted in an avalanche of mail asking that these recommendations be implemented. A letter of reply and a leaflet detailing Institute plans for implementation was sent in response to all Congressional and White House letters on behalf of the Commission.

Similar upswings in mail volume occurred as a result of the release of the NINDS report on the Research Aspects of the Neurological Bases of Aggressive Behavior and of the proposed gradual phasing out of the NINDS Information Network. Additional thousands of inquiries were answered with printed materials. In all, NINDS responded to requests for 263,982 publications.

The Office of Scientific and Health Reports interacts actively with the 20 national voluntary health agencies and some 20 professional societies which make up the National Committee for Research in Neurological Disorders. As part of the reorganization of the information activities, publication of the NINDS Review, a bi-monthly newsletter of interest to the neurological community, was assumed by the National Committee, renamed the NCRND Newsletter, and given a somewhat different orientation.

In addition to Institute and voluntary health agency news, the newsletter now also contains legislative items. The Office of Scientific and Health Reports continues to provide material on Institute research, programs, and personnel for the NCRND Newsletter.

Through an exhibits program in this Section, the Office of Scientific and Health Reports brings the Institute's extramural activities to the attention of the professional societies and the health agencies at national scientific meetings. Last year the Program Exhibit was shown at the ANA in Montreal, the AAN in Boston, and the AAOO in Dallas. All arrangements for scheduling and manning the exhibit were handled by staff of this section. These showings generated hundreds of requests for information about Institute programs.

Members of this section also keep abreast of Advisory Council meetings, plan the annual Council dinner, and update a Council Directory. Additionally, the head of this section is the liaison person with the extramural area and is responsible for providing grantee information for use in Institute reports and publications.

The Public Inquiries Section responded to 128 requests for summary minutes of the meetings of these groups and for lists of their members. The section also provided the Department with quarterly reports of this activity.

The Office of Scientific and Health Reports also serves as the focal point for the Institute in discharging its responsibilities under the Freedom of Information Act.

Scientific Publications Section

The Scientific Publications Section produces and distributes publications, both for the general public and for various scientific audiences. In the past several years, these services have been substantially increased. Publicatons production services are requested by various administrative units of the Institute, ad hoc committees preparing reports, and outside organizations in the neurological field if sufficient need is demonstrated. The services include planning, writing, editing, design layout, clearance, distribution, storage, and later revising and reprinting, according to demand. The Section works with the NIH Printing Unit, the Medical Arts and Photography Branch, and the Government Printing Office. It also serves as the Institute's supply and distribution center for all publications

The Monograph Series contains neurological science contributions of Institute staff members, committees, and consultants. Included are reviews, reports, nomenclature, classification and methodology aids, and proceedings of meetings sponsored by the Institute. The thirteenth in this series, "The Epidemiology of Epilepsy: A Workshop," was delivered in October 1973. This publication, containing some 28 papers with many tables and charts, has been sent to physicians, scientists, libraries, and other individuals and organizations.

Bibliographies are also produced and distributed by the Section. An NINDS Bibliography Series was started in Fiscal Year 1973 with bibliographies on Brain Death and Blood Level Determinations of Anti-epileptic Drugs; others will be produced at irregular intervals. These are computer-assisted compilations of the Institute's Applied Neurologic Research Branch. The Cerebrovascular Bibliography, a quarterly selection of stroke references from Index Medicus, was changed from a free distribution status (500 recipients) to a paid subscription system with the January-March 1974 issue. An Epilepsy Bibliography, covering the period from 1900 to 1950, was published in July 1973. An annual bibliography prepared by the Perinatal Research Branch is also handled by this Section.

The latest of the Profile Series, NINDS Research Profiles 1973, was published in March 1974. This is an 87-page booklet containing special Congressional reports covering research advances and an opening statement by the director on general program directions.

The Institute's highly popular Hope Through Research series continued to be revised and reprinted as necessary. Two new leaflets, on dystonia and myasthenia gravis, were written by office staff and will be published in Fiscal Year 1975. This will bring to 20 the number of leaflets in this series. The Hope Through Research leaflets, three of which are in Spanish as well as English, are the most widely circulated of the Institute's publications.

The National Advisory Commission on Multiple Sclerosis completed its report in February 1974. The Scientific Publications Section had a mimeographed edition of this report printed for immediate distribution, and began work on a typeset edition, which will be published in Fiscal Year 1975.

Other items produced by the Section included Information Resources for the Neurosciences; a directory of films, audiovisual aids, and abstract and bibliographic services; an updating of Research Grant Support in the Neurological, Sensory and Communicative Fields; a booklet for grant applicants; a folder for students entitled Where Can I Go For Information on the Brain and Nervous System; a newsletter for the EEO Program; and reprints of various publications still in demand.

The Section is now distributing 53 publications on neurological and sensory disease problems. Of these, 32 were written by office staff. The Section contributed editorial and production services for the remaining 21, which originated with scientific authors and groups. Altogether, more than a quarter of a million of these publications were distributed in Fiscal Year 1974.

Health Reports Section

The Health Reports Section is responsible for the preparation of Institute research and health reports for Congress, the scientific community and voluntary agencies. Some of these are written and others produced on film and video or audio tape. The Section is also responsible for arranging the widest possible distribution of the reports to the medical community.

Eight scientific lectures on neuromuscular disorders were videotaped in Houston in March. There are now 25 reports on this subject taped at the Houston Veterans Administration TV facilities, and seven specifically on the "Combined Clinical Staff Conference on Myasthenia Gravis" taped at the Naval Medical Center TV studios, a total of 33 lectures which the Section has produced and is distributing by loan or sale.

The Section assisted the Epilepsy Foundation of America, EFA, in filming interviews with four Institute scientists including the Director. Staff arranged and scheduled interviews, made appropriate filming sites available and assisted in camera setups and movements between sites. Later, three staff members and one person from the Audiovisual Branch, NIH/OD Information Office went to The University of Virginia Medical School, Charlottesville, Virginia, for two days and filmed (a) an interview with the physician there in charge of the epilepsy research program for the EFA film, and (b) sequences for a short news release or public service announcement planned by the Audiovisual Branch/OD in late summer.

The Government Printing Office used sales copy prepared by the Section in a flyer mailed to some 2.5 million persons offering the Institute booklet, "Discovering Yourself in the Brain Age" for sale for 75¢ a copy. Within a short period of time, GPO sold 10,000 copies of the booklet and went back for a reprinting of 7,000 more copies. As of April 1, they had sold 1,140 more. Public service radio and television announcements intended for release with GPO materials were cancelled before reaching quantity-production stages when decisions were made to use 16,500 copies of the original stock to interest science classes in junior and senior high schools in using the booklet to increase young people's interest in brain research. National Institute of Mental Health's Office of Communications personnel discovered the booklet and with NINDS agreement publicized it in their radio-spot and newspaper-column services.

Coordination between the Institute, the National Committee for Research in Neurological Disorders (NCRND), comprising some 20 voluntary health agencies and professional societies in the neurological and communicative area, and the National Science Teachers Association (NSTA) focused attention on brain research. The Institute is providing one copy of "Discovering Yourself in the Brain Age" to each NSTA member, and the science department head in almost every junior and senior high school in the country. Study materials are also included to convince these science departments that the booklet can be an excellent complement to other curricula materials for teaching science in the fall semester of 1974. It is expected that the effort will result in many more copies of the booklet being sold, that knowledge of the brain and brain research will be greatly increased, and that interest in pursuing neurological careers will be stimulated in thousands of young persons. The NSTA intends to feature the project in its official magazine, "The Science Teacher" before the fall semester begins.

The Section is conducting an office effort to bring Veterans Administration and Institute neurological scientists and television

groups together to identify needs which exist for improving professional education in neurology through use of television production and taping facilities and to plan ways that such VA and Institute facilities can help to fill the needs.

To make the Health Reports Section truly responsive to the needs of the Institute's laboratory chiefs, bench scientists and Director's executive staff, the Section is producing a short video-taped presentation about how it can serve their individual and collective requirements to speed communication of vital research information to other scientists, neurologists and general practitioners.

Table of Organization

Intramural Research

National Institute of Neurological Diseases and Stroke
(Personnel on hand May 1974)

Office of the Director

Director of Intramural Research - Henry G. Wagner, M.D.
Assistant Director of Intramural Research - Richard L. Irwin, Ph.D.
Act. Clinical Director - John M. Van Buren, M.D., M.Sc.
Administrative Officer - Glenn E. Hammond
Administrative Assistant - J. Loring Jenkins
Photographer (Scient. & Tech.) - Wesley Pearson, Jr.
Photographer (Medical) - Jerry M. Ellis
Administrative Assistant - John H. Jones
Secretary (Steno) - Margret L. Shipley
Administrative Technician - Doris R. Perry
Travel Assistant - Ida M. Chernikoff
Biological Laboratory Technician (Animal) - George R. Duvall
Biological Laboratory Technician (Animal) - Adrian P. Loftis
Biologist - Minnie Toure
Procurement Clerk - Patricia D. Williams
Budget Clerk (Typing) - Lillian Killgo
Clerk-Stenographer - Sylvia Levinson
Clerk-Typist - M. Jacquelin Concannon
Laboratory Worker - John R. Bowers
Laboratory Worker - Kenneth Oglesbee
Laboratory Worker - Richard Bynaker

Section on Technical Development

Computer Programmer - William H. Sheriff, Jr.
Engineering Technician - Benjamin H. Shellman
Staff Fellow - Bruce M. Smith, Ph.D.

Medical Neurology Branch

Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Biological Laboratory Technician (Microbiol.) - Guy G. Cunningham
Biologist - Joseph G. Brown
Physiologist - E. Carolyn Derrer
Biologist - Mary A. Oberc

Surgeon - Barry W. Festoff, M.D.
Surgeon - Barry Levin, M.D.
Surgeon - Mark S. Horwich, M.D.
Surgeon - Richard W. Price, M.D.
Surgeon - Michael Swerdlow, M.D.
Surgeon - John W. Griffin, M.D.
Surgeon - Jay D. Cook, M.D.
Surgeon - Adam N. Bender, M.D.
Surgeon - John L. Trotter, M.D.
Surgeon - David S. Zee, M.D.
Visiting Associate - Ryoko Tsukui, M.D., Ph.D.
Secretary - Lucy L. Riley
Microbiologist - Priscilla B. Chauvin
Secretary (Dict. Mach. Trans.) - Gertrude E. Wright
Biological Laboratory Technician - Gregory C. Zirzow
Clerk (Dict. Mach. Trans.) - Anne K. Lawrence
Histopathology Technician - Thelma R. Fletcher
Clerk (Dict. Mach. Trans.) - Ann M. Macker
Biological Laboratory Technician - Gregory Hubbard
Biological Laboratory Technician (Microbiol.) - Joseph S. Sciabbarrasi
Clerk-Typist - Beverly Williams
Laboratory Worker - Matthew P. Meadows
Visiting Fellow - Hussein Dessouky, M.D.
Visiting Fellow - Neelupalli Reddy, Ph.D.

Section on Clinical Applied Pharmacology

Super. Research Pharm. (Gen.) - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Biologist - Katharine L. Oliver
Secretary (Dict. Mach. Trans.) - Emma P. Dick

Surgical Neurology Branch

Office of the Chief

Chief, Surgical Neurology Branch - John M. Van Buren, M.D., M.Sc.
Medical Officer (Neuro. Surg.) - Choh-luh Li, M.D., Ph.D.
Research Psychologist (Clin.) - Paul Fedio, Jr., Ph.D.
Research Psychologist (Clin.) - Joseph Bossom, Ph.D.
Biologist - Rosemary C. Borke
Histopathology Technician - Martha L. Johnson
Psychologist - Ghislaine R. Frederick
Surgeon - Adelbert L. Albright, M.D.
Surgeon - Norman D. Peters, M.D.
Surgeon - Daniel B. Ahlberg, M.D.
Surgeon - John C. Madigan, M.D.
Surgeon - Leslie D. Cahan, M.D.
Surgeon - John C. Oakley, M.D.

Staff Fellow - Amiram Daniel, Ph.D.
Medical Technician - Vivian A. Betton
Clinical Psychologist - Christiane S. Cox
Clerk (Dic. Mach. Trans.) - Helen M. Andregg
Secretary (Typing) - Lillian L. Wease
Secretary (Steno) - Olga G. Williams
Biological Laboratory Technician (Microbiol.) - Charles E. Sartor
Clerk-Stenographer - Patricia Price
Clerk-Stenographer - Myrtle Sullivan

Section on Applied Research in Surgical Neurology

Medical Officer (Neuro. Surg.) - Ayub Khan Ommaya, F.R.C.S.
Visiting Scientist - Susumu Nakatani, M.D.
Biologist - Kenneth Rich
Biological Laboratory Technician (Animal) - Calvin S. Hawkins
Biological Laboratory Technician (Animal) - M. Arthur Banks
Biological Laboratory Technician (Animal) - Clifford A. Seay
Clerk (Dict. Mach. Trans.) - Lois A. Brown

Section on Neuroradiology

Research Medical Officer - Giovanni Di Chiro, M.D.
Sr. Staff Fellow - Mary K. Hammock, M.D.
Clerk-Stenographer - Doris Selkowitz
Biological Aid - Carlton Lampkins

Electroencephalography Branch

Office of the Chief

Chief, EEG Branch - Cosimo Ajmone Marsan, M.D.
Surgeon - Darrell V. Lewis, Jr., M.D.
Surgeon - Barry I. Ludwig, M.D.
Surgeon - Boris A. Vern, M.D.
EEG Technician - Rosemarie Schilling
EEG Technician - Martha Fair
Secretary (Dict. Mach. Trans.) - Mattie L. Jackson
Clerk-Typist - Tina McDaniels

Section on Clinical Neurophysiology

Surgeon - John S. Ebersole, M.D.
Surgeon - Robert A. Levine, M.D.
Biological Laboratory Technician - Stuart Walbridge

Developmental and Metabolic Neurology Branch

Office of the Chief

Chief, DMNB - Roscoe O. Brady, M.D.
Staff Fellow - John L. Everly, Ph.D.
Secretary (Steno) - Barbara W. Critzer
Secretary (Steno) - Sandra S. Costanzo

Section on Enzymology and Genetics

Research Chemist - Richard H. Quarles, Ph.D.
Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Sr. Staff Fellow - Peter G. Pentchev, Ph.D.
Sr. Staff Fellow - Peter H. Fishman, Ph.D.
Staff Fellow - John F. Tallman, Ph.D.
Biological Laboratory Technician (Biochem.) - George E. Mook

Section on Neurochemical Methodology

Research Chemist - Andrew E. Gal, Ph.D.
Biological Laboratory Technician (Biochem) - Frank J. Fash

Section on Clinical Investigation and Therapeutics

Associate Chief, DMNB - Anatole Dekaban, M.D., Ph.D.
Research Chemist - Dennis F. Cain, Ph.D.
Research Chemist - George Constantopoulos, Ph.D.
Medical Technician (Chem.) - Jan K. Steusing
Surgeon - Andrew W. Zimmerman, M.D.
Surgeon - Marvin Zelkowitz, M.D.
Biological Aid - D. Douglas Fratz
Visiting Fellow - Ken Fujitani, M.D.

Laboratory of Neuropathology and Neuroanatomical Sciences

Office of the Chief

Chief, LNNS - Igor Klatzo, M.D., M.Sc.
Visiting Scientist - Krystyn Renkawek, M.D., D.M.Sc.
Research Biologist - Margaret R. Murray, Ph.D.
Biological Laboratory Technician (Animal) - Willie Perkins
Biological Laboratory Technician (Animal) - Albert V. Cantu
Biological Laboratory Technician (Animal) - Melvin H. Carroll
Secretary (Dict. Mach. Trans.) - Virginia A. Masterson
Clerk-Stenographer - Patricia A. Bustin
Clerk-Typist - S. Joan Eskin
Visiting Fellow - Omeo Ito, M.D.

Section on Neurocytology

Research Physiologist - Milton W. Brightman, Ph.D.
Biological Laboratory Technician - Clara E. Mowry

Section on Functional Neuroanatomy

Medical Officer (Res.) - Thomas S. Reese, M.D.
Biologist - Avery D. Nelson, Ph.D.
Biological Laboratory Technician (Gen.) - Frank D. Nolan

Section on Cerebrovascular Pathology

Biological Laboratory Technician - Joseph T. Walker, Jr.
Surgeon - Frank G. Berson, M.D.
Visiting Scientist - Bogomir Mrsulja, M.D.

Section on Experimental Neuropathology

Medical Officer (Path.) - Jan Cammermeyer, M.D.
Biologist - Iris Fenton
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

Section on Cellular Neuropathology

Medical Officer (Research) - Henry deF. Webster, M.D.
Biological Laboratory Technician - Maureen F. O'Connell
Staff Fellow - Paul J. Reier, Ph.D.
Histopathology Technician - Gertrude Goping
Biologist - John R. Martin

Section on Cellular Neurochemistry

Research Biologist - Janet V. Passonneau, Ph.D.
Sr. Staff Fellow - Wesley D. Lust, Ph.D.
Staff Fellow - Joan P. Schwartz, Ph.D.
Biologist - Helen J. Osborne
Medical Technician - Sandra K. Crites
Clerk-Typist - Wanda D. Dickens
Visiting Fellow - Branislava Mrsulja, M.D., M.Sc.

Section on Neurocytobiology

Medical Officer (Res.) - Maria Spatz, M.D.
Biological Laboratory Technician (Microbiol.) - Madora E. Swink

Laboratory of Biophysics

Office of the Chief

Chief, Laboratory of Biophysics - William J. Adelman, Jr., Ph.D.
Super. Research Physiologist - Robert E. Taylor, Ph.D.
Reserach Biophysicist - Kenneth S. Cole, Ph.D.
Research Physicist (Gen.) - Richard Fitzhugh, Ph.D.
Research Physiologist (Neurophys.) - Daniel L. Gilbert, Ph.D.
Electrical Engineer (Instru.) - Leonard Binstock
Research Physicist (General) - Gerald M. Ehrenstein, Ph.D.
Research Physicist (General) - Harold Lecar, Ph.D.
Mathematician - John Shaw
Electronic Technician - Herbert A. Walters
Sr. Staff Fellow - Frederick Sachs, Ph.D.
Secretary (Steno) - Vernita Bergmeyer
Biologist - Allen D. Spencer
Clerk-Stenographer - Sandra J. Cotter
Biological Aid - Mark A. Holbert
Biological Aid - Sandra I. Dixon
Biological Aid - Norman Magid
Biological Aid - Vivian Sege
Visiting Fellow - Robert J. French, Ph.D.

Laboratory of Neurochemistry

Office of the Chief

Acting Chief, Laboratory of Neurochemistry - R. Wayne Albers, Ph.D.
Secretary (Stenography) - Shirley A. Burdette
Clerk-Stenographer - Michele A. Cahill
Clerk-Stenographer - Pamela A. Barnicoat

Section on Amino Acid Chemistry

Chemist - Oscar M. Young
Biological Aid - Stephen Marshall

Section on Enzyme Chemistry

Physiologist - Stephen Goldman, Ph.D.
Chemist - George J. Koval
Surgeon - Alan C. Swann, M.D.
Physical Science Technician (Chem.) - Eunice L. Summers
Visiting Fellow - Dou H. Jean, Ph.D.

Section on Physiology and Metabolism

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter
Surgeon - Walter Reichert, M.D.

Section on Neuronal Development and Regeneration

Medical Director - Lloyd Guth, M.D.
Physiologist - Herbert Yellin, Ph.D.
Biological Laboratory Technician (Microbiol.) - Janina D. Ziemnowicz
Surgeon - Andrew A. Zalewski, M.D.
SR HSO - George F. Creswell
Visiting Fellow - Tae Oh, Ph.D.

Laboratory of Molecular Biology

Office of the Chief

Chief, Laboratory of Molecular Biology - Ernst Freese, Ph.D.
Microbiologist - Peter Cooney, Ph.D.
Staff Fellow - Dorothy A. Stroup, Ph.D.
Staff Fellow - Anthony L. Rosner, Ph.D.
Clerk-Stenographer - Mary W. Taylor
Secretary (Steno) - Geraldine Bitango
Clerk-Typist - Jacqueline L. Law
Biological Aid - Tanya Brown
Biological Aid - Asela Russell
Biological Aid - Abraham Rosner

Section on Developmental Biology

Research Biologist - Elisabeth G. M. Freese, Ph.D.
Surgeon - Jeffrey L. Simmons, M.D.
Sr. Staff Fellow - Martin D. Diesterhaft, Ph.D.
Sr. Staff Fellow - Richard C. Henneberry, Ph.D.
Chemist - Enid M. Galliers
Chemist - Cheryl L. Marks
Staff Fellow - Yong Kyu Oh, Ph.D.

Section on Molecular Virology

Research Chemist - Robert A. Lazzarini, Ph.D.
Staff Fellow - Michael F. Murphy, Ph.D.
Chemist - Leslye E. Johnson
Biological Aid - Mark Lieberman
Visiting Fellow - Gudrun Stamminger, Ph.D.

Laboratory of Experimental Neurology

Office of the Chief

Chief, Laboratory of Experimental Neurology - William F. Caveness, M.D.
Visiting Associate - Hideo Ueno, M.D.
Visiting Associate - Hiroyuki Nakagaki, M.D.
Clerk-Typist - Marguerite Smiley
Medical Technician - Linda Sandhaus
Biological Laboratory Aid - Robert Rosenberg
Visiting Fellow - Yoriaki Yamashita, M.D.

Laboratory of Neurophysiology

Office of the Chief

Chief, Laboratory of Neurophysiology - Michelangelo G.F. Fuortes, M.D.
Electronics Technician - Anthony F. Bak
Staff Fellow - Richard Normann, Ph.D.
Biological Laboratory Technician (Animal) - William L. Beane
Secretary (Steno) - Johanna L. Barnett
Clerk-Stenographer - Susan A. Jacobsen

Section on General Physiology

Research Neurologist - Ludwig von Sallmann, M.D.
Chemist - Patricia A. Grimes
Surgeon - Elliott J. Simon, M.D.
Senior Staff Fellow - Daniel L. Alkon, M.D.

Section on Sensory Physiology

Medical Officer (Research) - Thomas G. Smith, Jr., M.D.
Visiting Associate - Jun Fukuda, Ph.D.

Section on Cell Biology

Research Biologist - Arnaldo Lasansky, M.D.
Biological Laboratory Technician (Microbiol.) - Julia M. Lohr

Section on Neuronal Interactions

Head, Section on Neuronal Interactions - Henry G. Wagner, M.D.
Senior Surgeon - John McReynolds, M.D.
Senior Staff Fellow - Ferenc I. Harosi, Ph.D.
Senior Staff Fellow - Peter D. Detwiler, Ph.D.
Senior Staff Fellow - Helga Stanbury, Ph.D.

Laboratory of Neural Control

Office of the Chief

Chief, Laboratory of Neural Control - Karl Frank, Ph.D.
Medical Officer (Res.) - Robert E. Burke, M.D.
Biological Engineer - Edward M. Schmidt, Ph.D.
Super. Research Psychologist - Herbert C. Lansdell, Ph.D.
Research Physiologist - William B. Marks, Ph.D.
Senior Surgeon - Frederick T. Hambrecht, M.D., Ph.D.
Surgeon - John V. Walsh, Jr., M.D.
Surgeon - Gerald E. Loeb, M.D.
Engineering Technician - George M. Dold
Electronics Technician - George F. Laird, III
Physiologist - Joan S. McIntosh
Electronics Engineer - Martin J. Bak
Secretary (Steno) - Dorothy M. Wilbur
Clerk (Dict. Mach. Trans.) - Gloria K. Hall
Clerk-Typist - LaJuan Lawson
Visiting Fellow - William Z. Rymer, Ph.D.
Visiting Fellow - Kendro Kanda, M.D.

Laboratory of Perinatal Physiology

Office of the Chief

Chief, Laboratory of Perinatal Physiology - Ronald Myers, M.D., Ph.D.
Research Chemist - Americo Rivera, Jr., Ph.D.
Research Psychologist - Shun-ichi Yamaguchi, Ph.D.
Biological Laboratory Technician (Animal) - Esteban Monell-Torrens
Biological Laboratory Technician (Animal) - William Rodriguez
Surgeon - Francis W. Gamache, M.D.
Surgeon - Howard Kirshner, M.D.
Surgeon - William F. Blank, M.D.
Chemist (Biochem.) - Carmen L. Freixas
Secretary (Steno) - Ethel K. Muntjan
Anthropologist - Elizabeth Ratliff
Biological Laboratory Technician (Biochem.) - James Cane
Biological Laboratory Technician (Animal) - Bernardino Santana

Laboratory of Neuro-Otolaryngology

Office of the Chief

Chief, Laboratory of Neuro-Otolaryngology - Jorgen Fex, M.D., Ph.D.
Secretary (Steno) - Elsie M. Walter

Laboratory of Central Nervous System Studies

Office of the Chief

Chief, LCNSS - D. Carleton Gajdusek, M.D.
Super. Research Microbiologist - Clarence J. Gibbs, Ph.D.
Medical Officer (Research) - David M. Asher, M.D.
Social Science Analyst - E. Richard Sorenson
Biological Laboratory Technician - Michael P. Sulima
Social Science Analyst - Judity B. Meyer
Microbiologist - Nancy P. Luber
Biological Laboratory Technician - Alfred E. Bacote
Senior Surgeon - Paul W. Brown, M.D.
Senior Surgeon - Lon R. White, M.D.
Veterinarian - Herbert L. Amyx, D.V.M.
Surgeon - Roger Traub, M.D.
Visiting Scientist - Louis Herzberg, M.D.
Staff Fellow - Ralph M. Garruto, Ph.D.
Secretary (Steno) - Marion F. Poms
Biological Laboratory Technician (Microbiol.) - Helena L. Gilbert
Biological Laboratory Technician (Animal) - Joseph Pazdersky
Microbiologist - Monica Ann Lewis
Secretary (Dict. Mach. Trans.) - N. LaDonna Tavel
Clerk (Steno.) - Olive E. Childers
Animal Caretaker - Harry Winpigler
Animal Caretaker - Ezra Shafer
Translator - Jose Figirliyong
Clerk (Dict. Mach. Trans.) - Linda Z. Poole
Animal Caretaker - Eugene D. Webster
Biological Laboratory Technician (Microbiol.) - Randolph Taylor
Student Trainee - Steven G. Ono
Biological Aid (Gen) - Robert Wismer
Animal Caretaker - Hubert Saville
Animal Caretaker - Leon Vance
Clerk - Typist - Brenda Johnson
Social Science Aid - Linda Watson
Health Technician - Ivan M'Baginatao
Biological Aid - Kathy Kinny
Biological Aid - Gregori Brown
Biological Aid - Nina Green
Biological Aid - Mary E. Hartman

Annual Report of the Scientific Director
of the
National Institute of Neurological Diseases and Stroke
July 1, 1973 through June 30, 1974

The Intramural Research Program of the National Institute of Neurological Diseases and Stroke conducts research related to the nervous system and its disorders in its laboratories and clinics in Bethesda, Maryland. The research staff uses both basic and applied creative approaches of a wide variety to investigate nearly every aspect of the normal and deranged nervous system.

Thirty-one scientific disciplines are represented by the staff of 110 doctorate level scientists which pursue 131 research projects with vigor and enthusiasm. The projects have attracted during the year many doctorate-level visiting scientists and guest workers, 33 who are now at work within the laboratories and clinics. As judged by scientific productivity and the laudatory recognition given by the national and international research communities, the Intramural Research Programs have been remarkably successful during the past year. Two hundred and eleven major research communications have been published in the major science journals. Many more abstracts and speeches have been presented before both domestic and foreign learned societies and research departments in universities and other research institutions. The Intramural Research staff eagerly looks forward toward continued and increased support of programs which will enable them to help solve the problems created by the neurological diseases. The expertise of the staff is unexcelled, their accomplishments are noteworthy, and their hopes are high that their efforts will ameliorate and eventually eliminate the disability and destruction caused by the neurological and communicative diseases.

The program planning activities of the Office of the Director have continued during the past year along the revised and expanded lines developed during the previous year.

1. The fiscal system CLEAR (Computerized Local Expenditure Accounts and Reports) previously developed here has been expanded to include a personnel data bank for the Intramural Program. The quick access formerly in use for fiscal data is now available for information about personnel. This improvement of CLEAR has significantly improved the operation efficiency of this office. This accomplishment is due mainly to the diligent and innovative efforts of the Administrative Officer and his staff.

2. Review and analysis for program planning for individual Laboratories and Branches has continued on a semi-annual basis. The internal review serves a more direct operational purpose than does the program review by the Board of Scientific Counselors.

Several organizational changes occurred during FY 1974 that benefit the Intramural Program:

In the Molecular Biology Laboratory, the Section on Regulation of Nucleic Acid Synthesis has been renamed the Section on Molecular Virology. A changing program emphasis and reorientation toward more research on viruses necessitated the change so that the organizational designation accurately reflects the function of the Section.

During this fiscal year, a major part of the Laboratory of Central Nervous System Studies was relocated from a temporary facility at the Patuxent Fish and Wildlife Service. The portion of the laboratory operation which required the use of large primates was relocated in part to the Gulf South Research facility in New Iberia, Louisiana and in part to an NIH Building at the same location. The smaller primate and other animal holding needs were relocated to the Frederick Cancer Research Center at Frederick, Maryland. The laboratory portion not related to animal holding was brought to Building 36 at the National Institutes of Health, Bethesda. The increased efficiency brought about by this relocation augurs well for more progress from the virus research programs of that Laboratory.

During Fiscal Year 1974, the Intramural Research Program established a new Laboratory of Neuro-Otolaryngology. An outstanding scientist has been recruited as Chief and he began the on-site organization of the Laboratory during November of 1973. Details of the resources made available and progress in recruitment are contained in this document within the Summary Statement of that Laboratory and are therefore not mentioned here.

Two other new Intramural Research units are in the planning stage and are expected to be established during FY 1975: A Laboratory of Neuroimmunology and a Laboratory of Neuropharmacology.

Some remarkable findings were made in the various Laboratories and Branches of the Intramural Research Program which use biochemical procedures as primary approaches.

The Developmental and Metabolic Neurology Branch has demonstrated for the first time that patients with inherited metabolic disorders can be significantly aided by the administration of exogenous enzymes. This Branch also discovered that an inherited disease can arise from a deficiency of an obligatory biosynthetic enzyme. All previously known metabolic disorders which include lipid storage diseases are caused by deficiencies in catabolic enzymes. This finding provides new approaches for research into a potentially large number of hereditary disorders of unknown etiology involving many tissues and the central nervous system in particular. This Laboratory also has had good success in purifying sphingomyelinase from human placenta and they expect to be able to initiate replacement therapy trial with this enzyme in patients with Niemann-Pick disease in the coming year.

Other investigations in this Laboratory led to the discovery of a myelin-specific glycoprotein and work is in progress on the nature and fate of the myelin glycoprotein in brain tissue from patients who had multiple sclerosis.

The Developmental and Metabolic Neurology Branch is a prime example of the fruitfulness of combining basic research expertise with clinical capability to accelerate progress in understanding and therapy of certain of the neurological disorders.

The Laboratory of Molecular Biology has continued to investigate cellular membrane transport and differentiation and also the structure and replication of mammalian viruses. A major finding is that lipophilic acids, including food additives, salicylates and antiseptics inhibit the transport of amino acids in bacteria, or their membrane vesicles, by uncoupling the transport system from electron transport. The same compounds inhibit growth on human cells and also change their morphology. The work has identified the chemical changes correlated with growth or form changes in human cells and has further found how the changes can be reversed. These findings have greatly advanced our knowledge about how food additives work and the work thus forms a basis for the development of other effective and usable compounds.

It has been found in this laboratory that the myxo-, paramyxo-, and rhabdo-viruses replicate differently from other better known viruses. Replication depends on a specific RNA polymerase that is distinct from all of the RNA polymerases of the whole cell and this unique polymerase represents a target for chemotherapeutic approaches. An inhibitor that would stop replication has been found, but unfortunately it was also toxic to intact cells. The Laboratory also investigates the possibility of the use of defective virus particles,

which are noninfective, to interfere with replication of an infective virus. These studies are new this year, but offer hope for further progress in therapy of virus induced disorders.

The Neurochemistry Laboratory has maintained the goal of explaining neural mechanisms in terms of molecular biology and defining the biochemical basis of intra- and intercellular communication systems, particularly in the central nervous system. The interests focus on the properties of cell membranes. Of particular achievement is the demonstration that certain cell enzymes are oriented in the plasma membrane so as to function as extracellular enzymes by acting on extracellular substrates. This finding along with other results has led to an attractive hypothesis that extracellular release of adenyl nucleotides may function as neuro-transmitters and regulate cellular permeability and excitability. The research on neuronal regeneration in the Neurochemistry Laboratory demonstrated that it is possible for neurons in homografted ganglia to not only survive but regenerate their cell processes and establish functional connections with end-organs. The finding that transplanted neurons can survive is encouraging to the long-term goal of obtaining functional regenerations in neural tissue. Investigations of the molecular mechanisms of active ionic transport has defined distinct sites where interaction occurs between the transport enzyme and the transported ions. The role of these sites with respect to phosphorylation and dephosphorylation have been described in detail. Using immunological methods, the laboratory has confirmed that two different large polypeptides constitute the major components of the transport ATPase. The uncovering of these detailed enzymic mechanisms explains at the molecular level the function of essential enzymes of the nervous system. The finding may have heuristic value in our understanding of all enzyme function.

Four laboratories in the Intramural Program use bioelectric recording as the primary, although not the only, technique for the gathering of information on nerve cell function. The Laboratory of Biophysics investigates the molecular and cellular mechanisms of nerve impulse generation and synaptic activity. Studies for five different types of giant axons are providing insight into both the molecular nature of the ionic channels associated with axon excitability and the action of neuropharmacological agents at the membrane level. The experiments performed by the Biophysics Laboratory are of the kind that will eventually lead to the final knowledge on how a nerve cell communicates with other cells. The laboratory findings during the past year are extensive, substantial and are succinctly summarized

in this report by the Laboratory Chief. Studies on ionic channels in post-synaptic, synthetic membranes, mathematical models, subcellular structures of nerve and muscle and on voltage clamp and membrane conduction have yielded much new information. In addition, the investigation of peri-axonal spaces on clefts has enabled the laboratory to predict the role of glial cells in epileptogenesis in the central nervous system.

In their search for knowledge of how the central nervous system connects functionally to sensory organs, the Laboratory of Neurophysiology conducts investigations on mechanoreceptors in vestibular organ, visual pigments, retinal cell interactions and degenerations, and the membrane properties of neurons. The electrical responses obtained from visual cones after their exposure to light have led to new interpretations of the color-dependency of the horizontal cells in the retina. Some animal models of retinal degeneration which resemble that found in human beings have been discovered. It is hoped that investigation of the animal model will help in understanding the causes of retinal degeneration in man.

The Laboratory of Neural Control functions under the herculean goals of describing the basic mechanisms of motor control of the central nervous system and developing techniques for utilizing these CNS control systems both by input and output for the benefit of the handicapped person and developing an awareness of the social consequences of their use. A major effort has been directed toward the development of techniques to transfer information both to and from the CNS. Such research necessitates knowing which materials are suitable for implantation into brain tissue. This Laboratory has made notable progress in electrode development. A new microelectrode called the "hat pin" electrode has been tested and by its use signals from a single cell have been recorded from animals for eight months. Groove electrode arrays have been developed which may allow recording from individual nerve fibers. A cable and barb electrode for recording from single motor units in muscle has also been developed. A major technical advance from this laboratory has been the design and development of a micromanipulator which can follow the movement of the cortex and is thus usable for introducing microelectrodes into the brain. The device is deemed ready for human trials. An important portion of the Laboratory of Neural Control activity centers on a neural prosthesis program supported outside Intramural Research by the Collaborative and Field Research effort. The program is productive and deals with feasibility and practical aspects of prosthesis development for the visually handicapped person.

The Laboratory of Neuropathology and Neuroanatomical Sciences has continued its investigations of pathological mechanisms operative in many of the debilitating neurological disorders such as stroke and the demyelinating diseases. They have studied the delayed impairment of circulation called the "no-reflow" phenomenon which occurs in areas of the brain subjected to ischemia. The new findings indicate a close relationship between the no-reflow phenomenon and fluctuations in systemic blood pressure. They further found that drugs which prevent a drop in blood pressure following release of carotid occlusion were effective in preventing the "no-reflow" occurrence. Other experiments on ischemic brain edema demonstrated that abnormal accumulation of fluid intracellularly is the primary mechanism of edema. Only later is there damage to the cerebral vessels and a leakage of serum that contributes to the edema.

During the last year the Laboratory added the capability of tissue culture studies. The first study undertaken is the facilitated transport of glucose and the effects hypoxia and carbon dioxide on glucose transport. The expertise in this area of study is high as are the expectation for gaining significant basic data on cerebral mechanisms. The neurochemistry group has established that events regulated by cyclic AMP assure that glycogen can be readily mobilized when energy stores are required and that the synthesis of glycogen is curtailed when an energy supply is not needed. Another Section in this Laboratory has applied the freeze fracture technique to studying the membranes of astrocytes. They found that where astrocytes face blood vessels, cerebral spinal fluid or large neuronal cell bodies, their membranes are strikingly specialized. These cells may thus transport or store substances exchanged between neurons and blood. By use of the freeze fracture technique this group has also had remarkable success in elucidating the important details of synaptic structures.

The Neuropathology and Neuroanatomical Sciences Laboratory has vigorously pursued a program of basic research that relates to the problems of the demyelinating diseases. An unusual biological test object has been discovered in the use of Xenopus tadpole and one Section of the Laboratory has developed a model test system that is now ready to test sera and cerebrospinal fluid from patients with multiple sclerosis. Since there is at present not any way to diagnose or treat multiple sclerosis, this research progress offers a fresh hope for advancing our understanding of multiple sclerosis or other demyelinating diseases.

The Laboratory of Experimental Neurology continues its pursuit of knowledge on the ontogeny of focal seizures, the delayed effects of x-irradiation on the central nervous system

of primates and the Vietnam Registry of head injuries during war. The first of these studies has revealed that early in life cerebral seizures are under the control of subcortical nuclear masses and that later in life the seizure propagation is controlled by the cortex. Seizure propagation is being defined by regional blood flow studies with the long-range goal of identifying the neuronal systems involved so that they may be chemically coded and studied in relation to seizure activity. The laboratory has established that structure changes after x-irradiation proceed from intracellular to local tissue and then to remote and generalized effects. The data so far collected is consistent with the occurrence of a cellular reaction induced by a newly-activated virus.

A remarkable finding in the Laboratory of Central Nervous System Studies during the past year is that the heredofamilial form of Jacob-Creutzfeldt disease is transmissible and of a virus etiology. Although most cases of this disease are sporadic, cases have been found in families which occur over several generations. From two such families the disease has now been transmitted to primates. This is the first time in man that a disease with apparent genetic determination may have been caused by a virus. These and the related studies have so broadened the picture of virus-dementia relationship that we now apparently refer to transmissible virus dementias. The LCNSS program thus has been extended to a study of dementias of all sorts in an attempt to determine whether there is a virus etiology.

Another major effort of this laboratory is the final characterization of the molecular nature and structure of the atypical viruses now known to cause neurological disease. This problem is of immense importance in microbiology and medicine since it represents a new type of transmissible agent capable of producing disease in the nervous system. The study of disease patterns in primitive cultures and population isolates was the initial step in the important discovery of the virus origin of some neurological disorders and such studies continue as a necessary component in the laboratory's program.

In their studies on speech and memory the Branch of Surgical Neurology has found that the immediate memory is linked with the anterior temporal lobe of the brain and the hippocampal complex, whereas the posterior temporal and parietal cortex may support longer term memory. They have also described that amnesia and dysphasia are inseparable during cortical but not during subcortical thalamic stimulation. Collaborative studies with an outside Institute have determined that cortical lesions of the left hemisphere affect learning of auditory information while lesions of the right cortex influence visual learning.

Laboratory studies of trauma to the central nervous system have confirmed in larger primates that rotational motion is critical in the production of diffuse brain lesions while contact or the translational movement of impact is crucial for the induction of focal lesions. Animal model studies in the brain tumor program have shown that immunotherapy alone is a totally ineffective treatment and that a combined treatment of immuno-chemotherapy was not better than chemotherapy alone. Patients with malignant gliomas treated with immunotherapy had recurrences of their tumors earlier than patients receiving chemotherapy or the combined treatments. Long-term projects within the Branch also deal with dyskinesias, epilepsy and vascular diseases of the CNS and their treatment by surgical procedures.

In addition to its research activities, the Electroencephalography Branch has continued to provide a Clinical Diagnostic Service for each of the Institutes with clinical research programs conducted in the Clinical Center. About 1,300 referrals have been supplied. The Branch has succeeded during the last year in defining the relationships between potassium levels, electrical activity and oxidative metabolism during epileptogeneses in the hippocampus. Similar relationships seem to exist during cortical arousal. The metabolic changes were found to be at least as great during arousal as during the epileptogenic activity. These experiments and others of equal elegance and difficulty help in our understanding the basic mechanisms of the epileptogenic processes which form the basis of epileptic disease. A long-term study of epileptic patients who have undergone surgical treatment has revealed that the proper evaluative period is the first one or two years after treatment. The cure or improvement rate was higher after removal of medial structures of the temporal lobe. Removal of the lateral portions was less effective. These studies are soon to be published and are destined to be an important contribution to the surgical treatment of epilepsy.

The research of the Perinatal Physiology Laboratory in animal mammalian systems has established that insults during early fetal life can induce diverse kinds of neurological abnormalities. They have found also that asphyxia of newborn mammals can produce four distinct patterns of brain damage depending on the nature and severity of the asphyxial episode. Recent research from the laboratory has described the vascular mechanisms of fetal death or expulsion and that such mechanisms can affect also the maternal structures. Other studies have defined that the depth of asphyxia which produces brain injury is close to that which produces death. Additional important experiments in the laboratory identified a major role for the

sympathetic nervous system with respect to the fetal status in utero. Excessive maternal sympathetic stimulation including that brought on by maternal psychological stress may cause fetal brain injury or death. An important adjunct to the brain damage studies are the investigations on drugs and toxic substances which may affect fetal life.

During this year, the research efforts of the Medical Neurology Branch have produced some notable progress. The hypothesis that Duchenne type muscle dystrophy is caused by ischemia mechanisms has excited other groups away from NIH who are now exploring the concept. The animal model developed in the Branch is now used for the screening in prophylactic drugs, and some potentially useful compounds have been uncovered. The Branch has also developed histochemical methods that can identify clinically normal carriers of this kind of dystrophy.

A new method that offers much promise has been developed for the diagnosis of the polymyositis/dermatomyositis disease complex.

Although discovered and introduced by this Branch sometime ago, it is noteworthy that the long-term acetazolamide prophylaxis and treatment of hypokalemic periodic paralysis is now being described in medical textbooks as the treatment of choice.

A new syndrome "levitated arms" has been described by the Branch and a successful surgical treatment was devised after discovery that the condition was caused by iatrogenic intramuscular injections in the deltoid muscle. Although not many cases have been described, the finding indicates a need for caution against repeated intramuscular injections into the same muscle.

In an animal model the Branch has demonstrated an adverse interaction between corticosteroid and anticholinesterase drugs. This finding may be of importance in relation to the modern therapy of myasthenia gravis. With this disease it has been found that lactate infusion serves as an apparent specific diagnostic test. The current research led to the postulate that lactate is a contributing factor in the excess fatigability of myasthenia patients.

The Medical Neurology Branch has reported a new disorder; family sensory peripheral neuropathy with elevated serum IgA. They have also demonstrated that idiopathic amyloidosis is often the cause of undiagnosed sensory neuropathy. Neurological abnormalities were also found in a number of patients with primary hyperparathyroidism. Three new disorders that reflect an abnormal neural influence on muscle have been described: 1) a new form of central core disease; 2) a disorder involving

focal loss of cross striations in muscle and 3) a new form of benign congenital hypotonia.

During the past year the Intramural Research group conducted 131 active projects. Twenty-eight new projects were begun. Three projects were completed and 24 were terminated. Thirty-four projects were in active cooperation with units in other NIH Institutes. In all there were 103 collaborating Institutes outside NIH. Ten scientists from the Intramural Program served as Project Officers on projects carried out in foreign countries under support by the PL-480 programs. Intramural has had 23 foreign or visiting scientists working in its programs at the end of the year, either in the guest worker or visiting category. These extensive interactions with other research efforts and scientists attest to the marked influence of the Intramural Program upon both the domestic and foreign research communities.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological Diseases and Stroke

July 1, 1973 - June 30, 1974

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer scientists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINDS. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art design techniques and components.

(3) Computational services. The Section assists the investigators in data collection, reduction, and analysis, by supporting three laboratory-size digital computers for general use, a medium-size time-shared digital computer (SEL 810B) for real-time on-line applications, and by providing programming service and technical consultation.

Additional services provided by the Section include consultation on: measurement techniques; signal processing; noise and electro-magnetic interference in data measurement systems; and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

INSTRUMENTATION

Following are selected instrumentation projects completed by the Section during the past year. These are chosen from a total of 194 projects, many of which consisted of routine fabrication of electronic circuits and mechanical devices.

(1) Telemetry System for Monitoring Patient Movement. A system was developed to monitor the activity of subjects remotely as they move freely within a clinical ward. The detector is built in the form of a wristwatch, and movement of the subject's arm causes a ball bearing to roll in a circular raceway and change the frequency of transmission. The amount of change is proportional to the intensity and rate of subject movement. The transmitted signal is received on antennas distributed around the ward, demodulated, and compared to an adjustable threshold. If the subject's motion is sufficiently intense, a standard pulse is produced which indicates one unit of motion. These pulses are counted and totaled every 10 minutes. The totals give an indication of the subject's daily activity cycle.

(2) Poolesville Rat Colony Data Acquisition System. This system has the capacity to collect, format, and store on tape, in real-time, data from 512 digital input channels. Data is in the form of (a) rat lever-pressing epochs, and (b) a digital number representing the identification of a particular rat passing a particular sensor, and its direction of movement. The system incorporates a computer, tape recorder, and extensive digital logic. The various I/O handler and driver software for the system's peripheral devices were also developed by the Section, as was a software package to model system operation in real-time.

(3) Instrumentation for Acupuncture Research. A stimulator was developed to deliver bipolar rectangular current pulses through acupuncture needles. The amplitude and frequency of the pulses are adjustable, and buffered outputs allow identical current pulses to be passed through ten needles. Currently being developed is a skin impedance mapping device for the location of acupuncture points. Numerous wire electrodes are placed in contact with the skin, and the impedance between each and a reference electrode is measured, to test the theory that acupuncture sites have lower impedance than immediately adjacent points.

(4) EMG Biofeedback System. This instrument allows a subject to learn to relax specific muscles by using the technique of biofeedback. The subject applies skin electrodes and the electrical activity of the selected muscle modulates the amplitude and frequency of an audio tone. The modulating voltage, proportional to the rectified, filtered EMG, is also displayed on a meter, giving the subject both auditory and visual feedback.

(5) Isometric and Isotonic Reflex Measuring System. This apparatus was developed to allow the measurement of the Tonic Vibration Response (TVR)

of the triceps surae muscle group in human subjects. This response is elicited by mechanical vibration of the Achilles tendon and the resulting plantar flexion is measured isometrically by recording the force developed against a stationary footplate or isotonically by recording the amount of rotation about the ankle joint while moving against a constant force. Both prone and supine subject positions may be used.

Owing to its versatility, this apparatus will be used to measure the TVR before, during, and following the implantation of a cerebellar stimulator in human epileptic patients. The measured responses will be used to study the effect of cerebellar stimulation on mono- and polysynaptic reflex pathways at the spinal cord and brainstem level.

(6) Amplitude and Time Window Discriminator. This instrument was developed to detect specific neurophysiological signals in the presence of noise and other signals. For discrimination, a signal must remain between an upper and a lower amplitude bound for longer than a specified minimum time, but less than a maximum time. Use of the two-dimensional window results in greater noise reduction and more accurate discrimination. A multiplexing system allows the data signal, the two amplitude window levels, and the discriminated pulse to be displayed simultaneously on a single oscilloscope beam.

(7) Ion-Sensitive Electrode Amplifier System. The system is comprised of two instruments: an ultra-high input impedance amplifier and filter combination for use with ion-sensitive electrodes; and an ion activity computer which generates the antilog of the amplifier output and gives an output proportional to ion activity over the range 1mM to 10mM.

(8) Infant Motion Detector. This apparatus automatically records infant arm-reaching and leg kicks in a mother-infant mutual learning study. Two infrared light source-detector pairs are mounted on adjustable parallel arms; when infant motion breaks a light beam, a binary signal is generated. Infrared sources were used to avoid distracting the infant with visible light. Focusing at the source and detector allows a separation of five feet, thus keeping the apparatus beyond the infant's reach.

COMPUTER SERVICES

The following table shows the utilization of laboratory computers.

	NIMH	NINDS	NICHD	TOTAL HOURS
MicroLINC-300	64%	36%	--	1408
PDP-12	17%	77%	6%	3428

The unusually heavy utilization of the PDP-12 was a result of the Applied Neurological Research Branch, C&FR using the computer evenings and

weekends. New programming techniques have made the PDP-12 easier to use and the anticipated work load will remain high, even after delivery of ANR's new computer.

The prime users of the microLINC have converted to their own computer systems and potential new applications are being evaluated. The Classic LINC has become unreliable, and as a result, was used less than 200 hours; it will be surplused as soon as is practical.

The SEL 810B computer installation in Bldg. 10 continues to operate reliably, routinely supporting three or four simultaneous users in on-line research applications. Support for this installation is now limited to a routine level of one man year for the systems programmer and less than one-half man year for new interface requirements.

ESTIMATED SEL 810B UTILIZATION

	Total Hours
Laboratory of Psychology, NIMH	
Section on Higher Thought Process	400
Section on Perception	50
Unit on Psychophysiology	1250
Adult Psychiatry Branch, NIMH	
Section on Psychiatric Assessment	150
Section on Experimental Group and Family Studies	100
Section on Perceptual and Cognitive Studies	1500
Laboratory of Clinical Psychobiology, NIMH	
Section on Comparative Studies	150
Laboratory of Socio-environmental Studies, NIMH	
Section on Personality and Environment	175
System Development	200
TOTAL	3975

ENGINEERING AND FABRICATION

The final table shows the distribution of the Section's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Adult Psychiatry, NIMH	3411	12.2
Brain Evolution & Behavior, NIMH	2979	10.66
Psychology, NIMH	2814	10.07
Neurophysiology, NINDS	2450	8.77
NIMH 810B Computer	2354	8.42
Neuropharmocology, St. E's, NIMH	2004	7.17
Clinical Science, NIMH	1636	5.85
Behavioral Biology, NICHD	1328	4.75
Biophysics, NINDS	1045	3.74
Molecular Biology, NINDS	897	3.21
Technical Development, NIMH/NINDS	868	3.10
Clinical Psychobiology, NIMH	776	2.77
Neurobiology, NIMH	764	2.73
Surgical Neurology, NINDS	749	2.68
Neurophysiology, NIMH	719	2.57
Neural Control, NINDS	508	1.82
Neurochemistry, NINDS	468	1.67
General & Comparative Biochemistry, NIMH	402	1.43
Neuropathology & Neuroanatomical Sciences, NINDS	362	1.29
Experimental Neurology, NINDS	347	1.24
Collaborative & Field Research, NINDS	290	1.03
Neurochemistry, NIMH	213	.76
Electroencephalography, NINDS	145	.51
All Other Labs	400	1.43
NIMH (Total)	18,506	66.23
NINDS (Total)	8,108	29.02
NICHD (Total)#	1,328	4.75
TOTAL*	27,942	100.0

*The time of the Section Chief is not included in the foregoing totals.

#NICHD loans the Section one position, and is thus entitled to 2000 hours of service.

ANNUAL REPORT
1 July 1973 through 30 June 1974
Report of Acting Clinical Director
National Institute of Neurological Diseases and Stroke

J. M. Van Buren, M. D., Ph. D.

The clinical program of NINDS is supported by four Branches, A. Electroencephalography and Clinical Neurophysiology (EEG), B. Medical Neurology (MN), C. Surgical Neurology (SN), D. Developmental and Metabolic Neurology (DMN).

The following summary is a synthesis of the programs of the various Branches, arranged by disease topic rather than origin of the research although the latter is indicated by appending the initials of the Branch. Further material can be obtained by reference to the Annual Report provided by the particular Branch.

The Intramural Clinical Program is a multifaceted effort and the studies have been arranged under the following headings:

I. Studies of the Developing Nervous System
II. Metabolic Disease of the Nervous System
III. Epilepsy
IV. Cerebrovascular Disease
V. Tumors
VI. Neuromuscular Disease
VII. Function Studies of the Human Nervous System
VIII. Neural Trauma
Statistics of the NINDS Clinical Service

I. Studies of the Developing Nervous System

A. A myelin-specific glycoprotein (DMN)

Our investigations of the myelin-specific glycoprotein, which was discovered in our laboratory, have been extended in several directions. We have found that the size of this novel myelin protein decreases from a mass of 130,000 daltons to 110,000 daltons as myelin matures. We are actively investigating the nature of the 20,000 dalton fragment which is cleaved in the course of normal myelination. We have evidence that this process is essential for the maturation of myelin since the residual myelin in myelin-deficient Quaking mice contains the higher molecular weight glycoprotein and compaction of myelin is impaired in these animals. Jimpy mice with virtually no myelin have none of the myelin glycoprotein in their central nervous system. This finding provides substantial evidence of the uniqueness of the glycoprotein to the myelin sheath. Additional work is in progress on the nature and fate of the myelin glycoprotein in brain tissue obtained from patients with multiple sclerosis.

B. Arhinencephaly (DMN)

Clinical and pathological studies on arhinencephaly were carried out on 53 patients whose brains were available for detailed histological studies. This most interesting of the congenital malformations of the brain is characterized by a wide spectrum of severity, ranging from simple absence of smell to the rudimentary brain, not compatible with survival over a few months of age. Three types were defined and criteria for diagnosis and classification established. The teratogenetic period was narrowed to 4-6 weeks of gestation and the expression of the severity was linked to varying doses of deleterious or genetic factors.

II. Metabolic Disease of the Nervous System

A. Lipid storage diseases

1. Classification (DMN)

Utilizing clinical, histochemical, chemical and electron microscopic studies in 13 patients with cerebral lipidoses, a new comprehensive classification was established and electron microscopic criteria outlined for the three different types.

2. Enzyme replacement (DMN)

It was demonstrated that administration of purified glucocerebrosidase isolated from human placental tissue to patients with Gaucher's disease causes a dramatic reduction in the quantity of accumulated lipid in the liver of these patients. Furthermore, the elevated level of glucocerebroside in the erythrocytes of Gaucher patients returned to normal following a single injection of glucocerebrosidase. The erythrocyte glucocerebroside has remained at the lowered level for over 4 months in these individuals. These demonstrations coupled with our previous work on enzyme replacement in Fabry's disease constitue the first definitive evidence that patients with inherited metabolic disorders can be significantly aided by administration of exogenous enzymes.

B. Mucopolysaccharidoses (DMN)

Detailed study of the composition of brain mucopolysaccharides and lipids in 7 patients who died with the diagnosis of one of various types of mucopolysaccharidosis permitted novel interpretation of the varying morbidity in different types of the disease.

It was demonstrated that Type V mucopolysaccharidosis consists of two enzymatically different categories of patients; certain of these patients have virtual absence of α-L-iduronidase whereas others have normal activity of this enzyme.

C. Menkes disease (DMN)

Investigation of abnormal copper metabolism in a hitherto lethal condition known as Menkes disease was carried out. Utilizing ^{67}Cu, we have demonstrated that only a fractional amount of oral copper is absorbed, but that the absorbed

copper is retained three times longer than in normals; the biological half-life of the copper in these patients is four-fold increased and little is released from the liver into plasma. However, red blood cells contain low normal complement of the copper. To overcome absorptive block, we have systematically studied various copper conjugates; the tri-sodium nitrillo acetate-copper conjugate proved promising in the therapy of this disorder. Results will be presented at the forthcoming meeting of the American Neurological Association.

III. Epilepsy

A. Pathogenesis (DMN)

It was shown that in brain lesions associated with marked increase in glial elements, certain protein, designated as "band 15", is greatly increased. This protein has been isolated and is in the process of being characterized; it appears to be the same as fibrous glial protein of Eng.

B. The animal model of epilepsy

1. The chronic alumina cream lesion (SN)

Review of the alumina cream macaque epileptic model has reemphasized the difficulties of obtaining a "mirror focus." Studies are planned to utilize this material in physiological evaluation of the inhibitory surround of the epileptic focus and an anatomical study of the morphology of epileptic foci and propagating pathways through histological techniques demonstrating significant stages in epileptic metabolism.

2. The 3-mercaptopropionic acid model (SN)

Studies of synaptic function and morphology are being undertaken in the 3-mercaptopropionic acid rat epileptic model and in human operative epileptic samples.

3. Surface monitoring of NADH levels (EEG - SN)

Basic studies carried out by N-EEG have indicated in the penicillin model, at least, that NADH fluorometric recording will differentiate between the primary and the projected epileptic activity. Technology is being pressed to make this apparatus available for real time use in the operating room. Initial experience in the human operating room is being reviewed using the earlier models of the apparatus.

C. Clinical studies

1. Medical therapy (DMN)

a. Anticonvulsant metabolism

Investigation of anticonvulsant levels in body fluids and the metabolism of these drugs in 2 groups of patients with epilepsy - children and adults - established the presence of significant differences in the turnover and katabolism of these compounds. Another finding of importance is presence of renal threshold for primidone, indicating that the commonly administered dose of this drug is much too high and that the amount given per kg body

weight/day will have to be reduced. We have established the ratios of phenobarbital in CSF:serum as 1:1.1, of primidone as 1:2.2 and diphenylhydantoin as 1:5.6.

b. Medroxyprogesterone

Value of medroxyprogesterone in control of seizures in catamenial epilepsy was demonstrated.

2. Surgical therapy (SN - EEG)

a. Late follow-up on the surgical treatment of temporal lobe epilepsy

The late follow-up results of 143 temporal operations for epilepsy have been evaluated at 3-18 years follow-up. The need for the long period of follow-up is strongly emphasized as well as the demonstration of a number of criteria which bear upon the surgical technique in the prognosis.

A special study of patients with combined bitemporal and temporal extratemporal foci demonstrated a reasonably hopeful outlook for these patients with temporal lobectomy which had not previously been suspected.

b. Cerebellar stimulation in the control of chronic epilepsy

The equipment is now available for current controlled transcutaneous stimulation of the human cerebellum in the control of chronic, intractable cerebral seizures in man. A pilot study of 5 cases of such implants is being initiated.

c. A practical means of extracellular recording from human cerebral cortex at open operation

In collaboration with N-LNLC a technique is now available which promises to provide stable recordings of extracellular cortical activity under operating room conditions. This offers an opportunity to further evaluate the chronic human epileptic focus.

IV. Cerebrovascular Disease

A. Pathogenesis (DMN)

Significant progress in the definition of current study on the pathogenesis and prevention of cerebrovascular disease was made. This project will continue to be "open-ended" until we are completely satisfied with the methods to be used. An important preliminary finding during the past 8 months: 12 out of 37 patients with stroke studied had excessive plasma lipid levels and 9 of these 12 had abnormal plasma lipoprotein. Admittedly, these particular patients may represent some selection bias, and, clearly, further studies and especially therapeutic modification are needed.

B. The animal model (SN)

A correlative study between sequential radionuclide brain scanning and time lapse microangiograms has been completed. In the majority of animals brain scintigraphy became positive by 2 weeks, to regress toward negative by 4-6 weeks after ligation of the middle cerebral artery. The increased isotope

uptake in the affected area was clearly related to neovascularization around the area of the infarct as shown by the microangiogram. Decreased vascularity, peripheral gliosis and central cavity formation are the main factors determining the diminution of the radionuclide penetration in the involved area at later stages.

C. Angiographic complications (SN)

Following clinical observation of postangiographic spinal cord damage, a primate model with a definite threshold of production of postangiographic paraplegia is being established. This model will be useful in testing various types of new contrast media. Evoked spinal cord potentials will be used to determine the point of early cord damage. In addition, an EM study will be made of the damage to the vascular tight junctions.

V. Tumors

A. Oncogenesis (DMN)

Cells transformed with Moloney sarcoma virus, a tumorigenic RNA virus, show the same synthetic enzymatic defect as cells transformed with the tumorigenic DNA viruses cited earlier. Cells transformed with the Kirsten strain of murine sarcoma virus, another tumorigenic RNA virus, show a different metabolic lesion. These cells are deficient in a galactosyltransferase required for the conversion of Tay-Sachs ganglioside to the next larger homolog. A similar enzymatic block has recently been observed by us in cells which are transformed by chemical carcinogens or by X-irradiation.

Additional research along this line in collaboration with investigators in LMB, IR, NINDS has provided an experimental system in which changes in the morphology of cells and ganglioside formation can be induced by the addition of salts of short chain fatty acids to the tissue culture medium. This ability to cause the switching on of a ganglioside synthesizing enzyme under carefully controlled experimental conditions provides a long-sought system for investigating the regulation of membrane lipid synthesis and is a unique opportunity to examine the correlation between the composition of complex lipids of cell membranes and morphological features.

B. The chemo-immunotherapy of the malignant glioma (SN)

1. Basic studies

a. The animal model has shown that preimmunization and chemotherapy are effective ways of treating the intracerebral murine tumor, but immunotherapy alone is a totally ineffective form of treatment. The survival of animals treated with immuno-chemotherapy was not significantly prolonged over that of animals treated with chemotherapy alone, although a reduction in the dose of chemotherapy required was suggested.

b. Histologically verified intracerebral delayed hypersensitivity reactions could be evoked in immune nontumor bearing primates with no significant neurological side effects when intracerebral PPD was given to

PPD skin-test positive animals. Increased phytohemagglutinin responsiveness of the animals' lymphocytes was also demonstrated in vitro suggesting that this enhancement of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumor immunotherapy. Further enhancement of PHA responsiveness was also demonstrated to nitrogen mustard and dinitrochlorobenzene, suggesting that such agents also may have adjunctive therapeutic value.

c. Studies continue to characterize the cell surface and to isolate the carcino fetal antigen which if successful would support immunotherapy as a powerful therapeutic tool.

2. Clinical studies

a. Diagnostic methods:

i. Cisternography: A study of normal CSF flow patterns with emphasis on the spinal CSF descent has been continued in the rhesus monkey and in man. The initial experience that failure of flow over the hemispheres may coincide with the location of an intracerebral tumor has been confirmed. There has been further experience in assessing the progression of neoplastic ventricular invasion, distribution of intrathecal medication and CSF shunts.

ii. Work has been continued on the Tomoscanner with changes in the geometry of the detecting probe and an indexer for tape records. These changes have improved both the resolution of the instrument and the ease of use. An extended clinical trial is planned.

iii. Initial experience is being gained with computerized axial tomography for brain tumors as well as of the hindbrain and spinal cord. In one instance it has been possible to resolve the central cavity in syringomyelia.

b. Therapeutic trial

i. Two patients with malignant glioma treated with immunotherapy alone had recurrences of their tumor before patients receiving chemotherapy or immuno-chemotherapy.

ii. A majority of glioma patients' lymphocytes are less effective in killing their own tumor cells than are lymphocytes of normal controls, irrespective of whether chemotherapy or immuno-chemotherapy is given after initial surgery and radiation therapy.

iii. Histologically, brain tumor biopsies from patients undergoing immunotherapy or immuno-chemotherapy, reveal lymphocytic infiltration of the leptomeninges and subpial areas, but no significant increase in numbers of lymphocytes within the substance of the brain tumors can be claimed because of the small amount of data and sampling errors due to biopsy limitations.

iv. To date the combined chemotherapy regimen, using oral CCNU and intratumoral 8-Azaguanine, appears to offer the best improved survival data (> 24 months for 50% survival, as compared to < 12 months for untreated controls).

VI. Neuromuscular Disease (MN)

A. Duchenne type of muscular dystrophy (DMD)

Further studies in clinically normal carriers of DMD have shown by a cluster of histochemical reactions on their muscle biopsies that subclinical muscle involvement can be identified.

B. Polymyositis/Dermatomyositis Complex (PM/DM)

A new histochemical finding - alkaline phosphatase staining in the connective tissue - can be diagnostically highly suggestive of PM/DM. The long-term high single dose alternate day prednisone treatment introduced some time ago continues to be the single best available treatment for this disease.

C. Late-onset rod disease

In two cases intranuclear rods, identical histochemically and ultrastructurally to myofibrillar rods in the cytoplasm, were found suggesting one of two possible new pathogenic mechanisms: (a) an origin from a previously unsuspected (or unemphasized) actin (or tropomyosin)-like, plus or minus alpha-actinin, contractile protein common to both nuclear and cytoplasmic sites, or (b) origin from cytoplasmic contractile protein imported into the nucleus and crystallized there into rods.

D. Myasthenia gravis (MG)

Treatment introduced here, LT-HSDAD-Pred, continues to be extremely beneficial in the majority of cases, 21 of 23, and for as long as 8 years in a child and 4 years in an adult. Lactate infusion has been demonstrated to be a new provocative and apparently specific test for MG patients. The binding site of the anti-muscle factor characteristic of MG (previously shown to be an IgG and true antibody) was localized in normal muscle with immunohistochemistry-electromicroscopy to the sarcoplasmic reticulum but only the SR and the I-band level.

E. Polyneuropathy (Peripheral Neuropathy) (PN)

Immunoglobulin survey of a large group of cases of PN currently considered "idiopathic" disclosed elevation of serum IgM in some, suggesting an immunologic pathogenesis in them. A new disorder, familial sensory PN with elevated serum IgA was reported. This was shown to be due to IgA overproduction in the small bowel tissue. This suggested possible familial susceptibility to infection in the gut tissue that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves.

F. Primary hyperparathyroidism

A neurologic abnormality was found in nearly every case. Most common

was involvement of the proximal limb and trunk muscles (attributed on the basis of histochemical and EMG studies to abnormality of lower motor neurons) sometimes with bulbar involvement, tongue tremors, and brisk reflexes. This gives an ALS-like syndrome, which reverts to or toward normal following surgical treatment of the hyperparathyroidism.

VII. Functional Studies of the Human Nervous System (SN)

The use of implanted electrodes for treatment and diagnosis and exposure of the brain with the patient awake during epileptic surgery, offer unusual opportunities for study of Speech, Memory and Cognitive function.

A. Limbic lesions

Research is concerned with neurophysiological and psychological changes following amygdaloid and cingulate lesions in man. Computer programs are currently being developed to assess the effects of surgery in changing the pain threshold of patients and to evaluate the effects of limbic lesions on personality and emotional status.

B. Cortical stimulation

In the studies of patients undergoing electrical stimulation of the exposed brain in the course of surgery for the relief of epilepsy, it was possible to map in the posterior temporoparietal regions of the left hemisphere distinct areas which support the ability to identify pictorial verbal material. Stimulation of the anterior cortical areas of the left temporal lobe and a wide area of the right brain failed to disrupt naming skills. Memory presses the anterior and posterior temporal regions into different support services. The primary or immediate memory system may be initially linked with the anterior temporal lobe and medial structures (hippocampal complex), while the posterior temporal parietal cortex may support secondary or long-term memory.

C. Depth stimulation

Perception and memory were studied during electrical stimulation with electrodes implanted in the lateral thalamus. Stimulation in the left pulvinar induced transient dysphasia and retrograde loss in recent memory for verbal memoranda. In contrast, comparable stimulation in the right pulvinar failed to disrupt verbal behavior and, instead, interfered with perception or recognition of complex visual patterns. Although the pulvinar nuclei behaved like related ipsilateral cortices with regard to general verbal or nonverbal functions, the cortical and subcortical stimulation results were different. Specifically, dysphasia and amnesia were indistinguishable effects during cortical but not during thalamic stimulation. Thus the contribution of the pulvinar and other posterior thalamic nuclei may be limited to pre-perceptual processing and may serve to shuttle sensory information to the cortical stations for final interpretation and analysis.

VIII. Neural Trauma

A. Basic studies

The crucial role of rotational loading for diffuse lesion production and of translational and contact phenomena components of impact for focal lesion production first demonstrated in the squirrel monkey are being confirmed, and in the larger rhesus monkey brain, permitting a closer analogy to man. Study of the effect of dynamic mechanical loading of frog sciatic nerve has shown a direct correlation between load intensity and amplitude of the compound action potential to compressive-tensile loads. The animal models are being used to develop trials of protective and therapeutic modalities for the amelioration of head injury severity.

B. Clinical studies (at D. C. General Hospital)

1. Severely injured patients with absent short latency waves in the visual and somatosensory evoked potentials on initial examination never recover full neurological function if these components show no recovery. It has been found that when short latency waves recover but subsequently disappear in a severely injured patient, a progressive and often surgically remedial lesion was found. Objective changes in a neurophysiologic assay, including evoked potential data, have been observed which correlate significantly with the symptoms of the posttraumatic syndrome.

2. In angiographic studies of spinal cord injuries, the sulcocommissural arteries have been demonstrated by high magnification in one patient. Extravasation of contrast medium into the epidural space has been shown in one case, indicating bleeding in progress. One of the tetraplegic patients who had been studied by angiography later died and it was possible to carry out a postmortem microangiographic study of the spinal cord. Thus it was possible to compare in vivo angiography and postmortem microangiography in the same specimen.

3. A predictive algorithm for head injury severity in man, based on our animal models, is being developed for use at the D. C. General Hospital.

Statistics of the NINDS Clinical Service
1 April 1973 thru 31 March 1974

Branch	Total OPD Visits	Total Inpatients	Total Inpatient days	Average Inpatient Stay	Remarks
Medical Neurology	789	319	5,884	18	410 Consults 400 Human muscle biopsies
Surgical Neurology	576	146	3,608	25	125 Neurosurgical procedures
Developmental & Metabolic Neurology	76	48	1,586	33	
Electroencephalography and Clinical Neurophysiology					1300 EEG examinations carried out

Serial No. NDS(I)-66-ODIR 1460
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Speech habilitation of the congenitally deaf--Fundamental problems and suggested directions

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To analyze the reasons for the general lack of success in speech habilitation of the congenitally deaf and to suggest areas of research which might contribute to progress.

Methods:

1. Review of literature.

2. Consultation with teachers of the deaf, and experts in allied fields--engineering, speech studies, and physiology, among others.

3. Analysis of problems.

Major Findings: The importance of interdisciplinary research, particularly the application of computer speech processing technology, stressed by this investigator since 1966, is becoming recognized. A program for research in many fields, presented in a paper "How can we teach the deaf to speak," hitherto unpublished was brought up to date and the paper accepted for publication in the A.G. Bell Association Quarterly Review, a new research journal to begin publishing soon.

Proposed Course of Project: This project has been discontinued because of transfer of the principal investigator.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68-ODIR 1554
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pure and applied mathematics, with special attention to mathematical models of sensory systems and of research institutions

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: To conduct research in pure and applied mathematics, with special attention to mathematical models of sensory systems and also of scientific research institutions. The latter will be directed toward improving the scientific management decision making process (systems analysis).

Methods Employed: Mathematical Analysis

Major Findings: An economical, easily operated computerized system (CLEAR) to record cost data in a form suitable for mathematical analysis is feasible for a relatively small research group (about 250 employees). Regular reports to management are a useful byproduct of such a system and greatly simplify day-to-day decision making.

Comparison of detailed costs over two years of operation show wide differences among Laboratories and Branches, but a surprising constancy for each Laboratory and Branch for the two years.

The methods of matrix algebra in conjunction with computerized cost data greatly simplify changing the basis of resource allocation and analysis, as for example from analysis by

organizational unit to analysis by program.

Proposed Course of Project: The project has been discontinued because of transfer of the principal investigator.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70-ODIR 1789
1. Office of the Director of Intramural Research
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Physical Senses

Previous Serial Number: Same

Principal Investigator: Edith L.R. Corliss, Physicist

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: Preparation of a publication summarizing and interpreting experimental results on the properties of the normal human senses of sight, touch and hearing. These intact human senses are to be considered quantitatively somewhat by analogy to the description and evaluation of measuring instruments, i.e., which mechanisms are optimized, what resolution limits are obtained, over what dynamic range do they function?

Major Findings: Survey of the current literature has continued, in order to obtain and interpret current experimental data on the performance characteristics of the intact human senses of sight, touch and hearing.

A survey of the experimental literature on loudness, undertaken in an effort to find a rational scale for the ear's response to intensity, led to the validation of a relationship first noted by R.R. Riesz in 1933. Riesz observed that listeners assess loudness as proportional directly to the intensity resolution limits of the ear. From recent literature, it turns out that over at least two orders of magnitude the loudness judgments and intensity discrimination functions track one another quite accurately. Thus the units of intensity discrimination are the measuring units for loudness.

This finding is not identical with the Weber-Fechner laws. According to them: $I/\Delta I$ = constant and loudness, L, is given by the relation: $L = \log_e I = \Sigma \Delta I/I$.

The relationships that are derived from the experimental data on the ear correspond to: $I/\Delta I \approx (I/I_o)^{1/4}$ where I_o corresponds to the sound intensity at the threshold of hearing, and the loudness of a sound of intensity I is given by:

$$\text{Loudness} = I/\Delta I$$

where ΔI is the difference limen for intensity observed for a sound stimulus of intensity I.

These relationships can be explained on a model that represents the ear as integrating a Rayleigh sum of the neural firings triggered by the sound stimulus (Corliss, 1967). The identical relations can also be derived from fluctuation theory with the ear represented as a statistical detector (Gabor, 1950). In either model, the same "least count" that fixes the threshold of hearing determines the intensity difference limen for the ear at all levels over which its response to a stimulus is free of saturation effects.

Inasmuch as the foregoing is a new verification of Riesz' hypothesis, which attracted almost no attention when it was proposed in 1933, a paper has been prepared summarizing the new results.

For the book, it is now possible to include intensity differential measurements with loudness measurements in an attempt to acquire a broader experimental basis for a set of equal-loudness contours describing the response of the ear to the intensity of stimuli.

If this relation can be found to hold also for persons with hearing impairment, it can be used as a basis for supra-threshold measurements of hearing function that are independent of linguistic background.

Proposed Course of Project: The remaining independent, free-standing papers on which work has been done, namely "Purely by Coincidence", "Interpretation of Concepts of the Critical Band" will be completed through editorial review. These will be reedited for inclusion in the latest draft of the section on hearing. In general, the preparation of free-standing papers has proved a slow and laborious process. It remains to be seen, from comparison of revised drafts with the original draft, how well this intermediate editorial process improves the ultimate quality of the writing in the book. Thus far, the writings have led to the development of new and original material that might otherwise not have been produced.

Efforts will be made to clear the following papers which are experiencing delays in various stages of editorial review: "The Recognition of Speech", "Channel Capacity of the Ear as a Function of Frequency", "Quantitative Consequences of Hearing Loss from the Standpoint of Communication Theory." Most delay has been encountered with the Editorial Review process at the National Bureau of Standards, which is slowed by stringent standards of accuracy.

Honors and Awards: None

Publications: None

Annual Report
July 1, 1973 through June 30, 1974
Medical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The current techniques consist of: histochemistry, clinical neurophysiology, biochemistry, immunology, electron-microscopy, and tissue culture. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic, electrical, and chemical definition of the abnormalities; (b) separation of each disorder into more distinct and often new sub-forms; (c) specific or symptomatic treatment; and (d) induced animal models closely related to the human pathophysiologic states.

For the clinical investigations, 319 patients were admitted for a total of 5,884 patient days, and there were 789 outpatient visits. There were about 400 human muscle biopsies processed histochemically. The neurologist provided consultation on 410 patients of other departments in the Clinical Center, performing indicated biopsies, myelograms, pneumoencephalograms, and electromyograms. In the past year, 12 papers were published, 16 are in press, and 21 were presented to meetings. One senior staff member has devoted full time to serving as Acting Assistant Scientific Director, IR, NINDS.

The one-year approved residency training program in neurology has been re-approved. Approximately 15 neurologists and other physicians and 12 technicians came this past year as guest workers to learn clinical research techniques in neurology, especially in neuromuscular diseases and the application of enzyme histochemistry thereto.

Many of our former trainees are full professors, associate professors, and assistant professors in academic departments, and many are directors of Muscular Dystrophy Clinics and Myasthenia Gravis Clinics; many are Medical Advisory Board members of the national Muscular Dystrophy, Myasthenia Gravis, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis Society Association/ Foundation.

The Department of Neurology, Warsaw Medical Academy is continuing its collaborative research program in neuromuscular disease with us under the PL-480 program.

Muscular Dystrophies and Other Non-Inflammatory Myopathies: Our histochemically-based hypothesis that the Duchenne type of muscular dystrophy (DMD) is possibly caused by ischemia mechanisms on the arterial side of the intramuscular vascular tree is now being explored by other groups. Having demonstrated in our model (rat aorta ligation followed by vasoactive amine) that following each dose of serotonin or norepinephrine the serum "muscle

enzymes" rise, as in DMD, we have used that parameter to screen for prophalactic drugs which then might be useful clinically to prevent muscle ischemic damage. Three such agents have been shown to prevent serum rise of muscle enzymes in the animal model -- dose-related in most instances but duel effects in one. In clinically normal carriers of DMD, we have reported that a cluster of histochemical reactions done on their muscle biopsies has proved very useful for identifying subclinical muscle involvement. A bizarre polysaccharide has been found as the storage material in muscle in a rare type of cardio-skeletal myopathy. In myotonic atrophy (myotonic dystrophy), several lines of evidence, based on our studies of patients and those of others, continue to support (or are harmonious with) our hypothesis that muscle involvement in this disease is not myopathic but due mainly to an altered nerve influence on muscle causing "fractionation" of motor units; therefore it should not be listed in the "dystrophy" category. In the pathogenesis of myotonia congenita and paramyotonia congenita, we have now postulated a major neurogenic aspect, which actually may be the pathophysiologic basis of the neuromuscular involvement, on the basis of our histochemical evidence harmonized with other patient-derived data.

Polymyositis/Dermatomyositis Complex (PM/DM): The finding of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases which we reported previously has now been confirmed by others. This supports our earlier hypothesis that childhood PM/DM may be another form of blood vessel abnormality in muscle, perhaps on the venous side or on both the venous and arterial sides. We have proposed that the detrimental immunologic mechanism might be different in the childhood cf. adult forms of PM/DM, immunoglobulin complexes cf. cell-mediated defects. Since the vascular and other immunologic abnormalities of childhood PM/DM could in turn be caused by a viral infection, search for Australian antigen, circulating or bound to blood vessels or muscle cells, was made but proved completely negative (with CC). Our attempts to "rescue" a virus from PM/DM patients' muscle thus far have been negative (with NCI and NIDR). Polytubular "myxovirus-like" structures were present in some capillary endothelial cells in PM/DM patients, but they are not disease-specific and their significance is uncertain. We will be reporting a new histochemical finding -- alkaline phosphatase staining in the connective tissue -- that diagnostically can be used as highly suggestive of PM/DM; it is especially evident in the childhood form. The method of treatment we introduced to this disease 6 1/2 years ago, long-term high-single-dose-alternate-day prednisone (LT-HSDAD-Pred), has continued to prove to be, in our hands (about 40 cases) and others, the single best available treatment for children and adults (without, or rarely even with, cancer). It has the greatest therapeutic benefit, fewest side-effects, and is easiest to manage. However, not all patients respond, the reasons for which we are seeking, as well as predictive parameters thereof. In non-responders we have successfully used azathiaprine combined with the LT-HSDAD-Pred.

Periodic Paralysis (PP): In the hypokalemic form of PP, the treatment we introduced, long-term acetazolamide (Diamox), has continued to be the best prophylactic agent both for preventing attacks and improving interattach weakness, and it is now in the textbooks as such. Two of our patients

have been treated successfully for more than 8 years. The mechanism of acetazolamide benefit in hypokalemic PP remains unknown, since muscle does not contain carbonic anhydrase. Renal calculi in one of 25 patients, possibly but not definitely related to the acetazolamide, have been the only suspected side-effects.

Other Myopathies: In two cases of late-onset rod disease intranuclear rods, identical histochemically and ultrastructurally to the myofibrillar rods in the cytoplasm, were found, suggesting one of two possible new pathogenic mechanisms: (a) an origin from a previously unsuspected (or unemphasized) actin (or tropomyosin)-like, plus or minus alpha-actinin, contractile protein common to both nuclear and cytoplasmic sites, or (b) origin from cytoplasmic contractile protein imported into the nucleus and crystallized there into rods. In one of those cases an associated unusual serum paraprotein, IgG/lambda myeloma protein, has been found. A new clinically delineated "levitated arms syndrome" has been described in 2 cases and the cause identified as iatrogenic intramuscular injections into the deltoids; one was successfully treated by surgical transection of fibrous bands. These cases demonstrate a need for caution against repeated intramuscular injections.

Neuromuscular Junction Abnormalities: The treatment we introduced to myasthenia gravis (MG), LT-HSDAD-Pred, has been confirmed and adopted by many other physicians. In our own series it continues to be extremely beneficial in the majority of cases, 21 of 23, and for as long as 8 years in a child and 4 years in an adult. Our only 2 non-responders were females in the menstruating age group. We have recently modified the treatment slightly by giving the single-dose 100 mg prednisone daily for the initial 2-3 weeks before converting to the alternate-day schedule, apparently resulting in more rapid improvement. For clarity of the initial study, anticholinesterase drugs were stopped prior to LT-HSDAD-Pred treatment and have not needed to be resumed in the vast majority of patients. Suspected from a few of our clinical observations in MG, an adverse interaction between corticosteroid and anticholinesterase drugs in an animal nerve-muscle test preparation has been reported. The possible degree and frequency of this animal-demonstrated adverse interaction requires cognizance and now further clinical evaluation in MG patients receiving both types of drugs. Lactate infusion has been demonstrated to be a new provocative and apparently specific test for MG patients; as a corollary, endogenous lactate has been postulated to be a contributing factor in myasthenics to their excessive fatigability as well as to their occasionally observed "Mary Walker phenomenon". The binding site of the anti-muscle factor characteristic of MG (which we previously showed was an IgG and true antibody), was localized in normal muscle with immunohistochemistry-electronmicroscopy to the sarcoplasmic reticulum but only the SR at the I-band level; its pathologic role, if any, remains mysterious. This localization also makes evident for the first time a structural-chemical specialization of the SR of the I-band cf. that of the A-band. With immunofluorescence we have demonstrated that a supposed "neuron-specific" nuclear binding factor of MG serum, as reported by others, is actually non-specific and simply a general anti-nuclear activity well-known in some patients with MG and other "autoimmune" diseases (but this has no bearing on our enthusiasm

for MG being a motor neuron disorder). Alpha-bungarotoxin was purified, isotopically labelled, and used autoradiographically to localize the acetylcholine-receptor molecules at the neuromuscular junction. Our NIH Clinical Staff Conference summarizing the pertinent ultrastructural, histochemical, physiological, pharmacological, clinical diagnostic, therapeutic, respiratory-care and nursing aspects of MG is in press (with NHLI and CC Nursing).

Polyneuropathy (Peripheral Neuropathy) (PN): Immunoglobulin survey of a large group of cases of PN currently considered "idiopathic" disclosed elevation of serum IgM in some, suggesting an immunologic pathogenesis in them. A new disorder was reported, family sensory PN with elevated serum IgA, shown to be due to IgA overproduction in the small bowel tissue -- on this basis a new hypothesis was introduced, one of possible familial susceptibility to infection in the gut tissue (in which there were found excessive eosinophils) that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves (with NCI). Our treatment of some cases of idiopathic PN with LT-HSDAD-Pred continues to be very successful and necessary, e.g., for as long as 9 years in an adult and 6 1/2 years in a child. Correlative studies indicate that patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, with elevated CSF protein, but even some patients with a dysneuronal type of PN, non-relapsing, or normal CSF have responded. With muscle biopsy histochemistry we have demonstrated that idiopathic amyloidosis is often the cause of a predominately sensory neuropathy in patients undiagnosed in other centers. Most were found to have immunoglobulin fragments in their urine and some to have an associated indolent myeloma. Treatment with alkeran has not been of obvious value. The PN of xeroderma pigmentosa was identified and reported, and its possible etiology speculated upon: it was hypothesized that the previously known defective DNA-repair mechanism might cause a shortened life in non-mitotic (neuronal) cells (enhanced neurologic "aging") because of inability to properly repair ordinarily banal neuronal DNA injuries (with NCI). Acquired pupillary dysautonomia (light-hyposensitive, tonic, mecolyl-hypersensitive) has been identified and shown to be a usually-overlooked but not infrequent (11 of 16 patients) accompaniment of idiopathic non-familial neuropathy, especially when there is a major sensory component (with NEI).

Motor Neuron Diseases: In amyotrophic lateral sclerosis (ALS), different approaches continue to fail to disclose the cause. For example, we have reported that a purported "toxic effect" of ALS serum on cultured motor neurons, published from another center, is not demonstrable in our laboratory. Histochemistry of anterior horn neurons in the cat was reported as showing a very rich machinery for anerobic glycolysis and a relatively poor one for oxidative metabolism in alpha-motor neurons in comparison with the other neurons of the anterior and posterior horns -- on this basis we raise one possibility that ALS might in some way involve defective glycogen metabolism. Benign focal amyotrophy has been described as a disorder simulating the lower motor neuron aspects of ALS at its onset but of spontaneously limited progression. In 16 patients with primary hyperparathyroidism we have reported that there is neurologic abnormality in nearly every case, the most common involvement being proximal limb and trunk muscle weakness (attributed on the basis of histochemical and EMG studies to abnormality of lower motor neurons),

sometimes with bulbar involvement, tongue tremors, and brisk reflexes: this gives an ALS-like syndrome, which reverts to or toward normal following surgical treatment of the hyperparathyroidism (with NIAMDD). In 10 cases of secondary hyperparathyroidism a similar proximal muscle weakness, also concluded to be caused by lower motor neuron involvement, has been found (with NIAMDD). In central core disease, further evidence was adduced by outselves and others to support our earlier hypothesis that it is more likely to be an abnormality of the neural influence on muscle than a myopathic disorder as originally proposed by others. On the basis of histochemistry and electronmicroscopy, three new disorders were identified and are in press, (a) a new form of central core disease in which there is a "hot spot" of enzymatic activity and lipid droplets in the center of the core, (b) focal loss of cross-striations, possibly due to defective motor neuron influence on muscle, and (c) type I muscle fiber smallness without central nuclei. A new form of the benign congenital hypotonia syndrome was discovered, consisting of marked type I muscle fiber predominance -- this was proposed to be caused by an in utero time-limited neuropathy affecting mainly the type I motor neurons. On the basis of integrated histochemical and electromyographic studies, a new hypothetical pathogenic mechanism was proposed, neurogenic "motor unit hypoplasia", to explain the type I muscle-fiber predominance we observed histochemically in cases of benign congenital hypotonia and central core disease, and in congenital rod disease and type-I-fiber-hypotrophy-with-central-nuclei too. In patients with non-neurologic localized (non-metastatic) cancer, before there is clinical evidence of anorexia, weight loss, or decreased daily activity, we have found significant type II fiber atrophy by muscle biopsy, at least some of which seems to be on the basis of defective lower motor neuron influence on muscle (with NCI). We are now attempting to analyse the mechanism in patients and trying to establish an animal model. This "distant effect" muscle weakness is often the most crippling aspect of cancer, and if the mechanism is known it might be treatable independent of treatment and response of the cancer itself.

Ophthalmo-neurology: In our series of about 30 patients we have demonstrated histochemically and electronmicroscopically that the commonest cause of progressive external ophthalmoplegia (after myasthenia gravis and myotonic atrophy are excluded) is the disorder characterized by "ragged-red" muscle fibers in limb muscles, whether or not the limbs themselves are weak. Other causes we have found are, (i) a vacuolar oculo-cranio-somatic myopathy, (ii) some of the cases of type I fiber hypotrophy with central nuclei, (iii) rare cases of lower motor neuron disorder, and (iv) rare cases of type II fiber smallness. Clinical and histochemical studies of oculomotor motor unit physiology, and of pupillary dysautonomia were also done (v.s., v.i.).

Central Nervous System Disorders: Spinocerebellar ataxias -- In two unrelated patients with Friedreich's ataxia, in whom we had previously shown impaired oxidation of pyruvate by their muscle slices, cultured skin fibroblasts have been shown to oxidize 1-^{14}C-pyruvate and 2-^{14}C-pyruvate at less than half the rate of (but U-^{14}C-glutamate at rates comparable to) those found in normal fibroblasts. This may be a metabolic defect that might help lead to the biochemical abnormality of at least some cases of Friedreich's ataxia. Epilepsy -- The new syndrome of familial myoclonus epilepsy,

limb-girdle dystrophy with "ragged-red muscle fibers," elevated blood lactate and pyruvate, and impaired oxidation of butyrate by muscle slices has been demonstrated. The ultrastructurally and biochemically abnormal mitochondria of the "ragged-red" muscle fibers (a now-widely-used light-microscope-derived term introduced by us to describe fibers containing abnormal masses of bizarre mitochondria) may provide a more accessible way of learning about the metabolic defect of the brain causing the epilepsy in this family.

Methodology and Basic Cellular Mechanisms: "Open-biopsy electromyography" (EMG) was developed by us as the first technique to give direct correlation of electrical activity of human muscle fibers during voluntary effort with the histochemistry of those fibers. It is done by taking the EMG apparatus into the operating room and recording at time of biopsy. Different physiologic firing patterns of type I and II neuron motor units were thereby identified: type I units fire as a slow sustained rhythmic discharge during slight effort, and type II units fire as limited bursts of rapid discharges only during greater effort. This demonstrates that in routine electromyography mainly or exclusively are measured the type I units for duration and amplitude, since measurement customarily is done during minimal voluntary contraction; and it necessitates modification in the way EMGs are interpreted. With the open-biopsy EMG technique it has further been reported that the pattern of brief-duration small-amplitude overly-abundant potentials (BSAPs) on voluntary effort can sometimes come from regions showing no myopathy in the usual sense but, instead, consisting of type I fiber atrophy, type I fiber hypotrophy with or without central nuclei, type II fiber paucity, and even "ordinary denervation" as in myasthenia gravis patients. From this we have proposed that the former and widely used designation of "myopathic EMG" for the BSAP pattern is erroneous, since according to our theoretical formulation it could also be due to (a) fractionation of the motor unit on an "ordinary" neuropathic basis or to (b) type-I-fiber (or both-fiber) smallness on a proposed subtle neurogenic defect-of-trophic-factor basis. If this proves correct it potentially alters the entire concept of the pathogenesis of a number of such diseases, in spite of the BSAP EMG pattern in them, including myotonic atrophy ("myotonic dystrophy"), myotonia congenita, paramyotonia congenita, central core disease, benign congenital hypotonia with I-fiber predominance, I-fiber hypotrophy, congenital rod disease, some cases of II-fiber atrophy, myasthenia gravis, the facilitating myasthenic syndrome, and possibly some cases of facio-scapulo-humoral syndrome. Even though we are writing and speaking that there is nonesuch animal as a diagnostic "myopathic EMG" many persons are still erroneously (in our view) using that term. We have developed an extracellular micromarking method for placing a spot about 50-100μ dia. in the human muscle biopsy at the time of open-biopsy EMG; the mark can then be displayed in histochemical sections to more precisely identify fibers adjacent to the recording electrode tip.

Tissue culture -- Cultured mouse embry motor neurons have been used for seeking toxicity of ALS sera (v.s.) and for beginning histochemical, electron-microscopic, EM-histochemical and scanning-EM studies. In the culturing of muscle biopsies from our patients, done in collaboration with Dr. Valerie Askanas (I.R.M., N.Y.U.), a new program has been developed for: (a) reliably obtaining luxurious growth in petri dishes by our special explant-

reexplantation technique (after the prepared tissue fragments are flown to New York), (b) achieving for the first time transverse-section histochemistry of cultured human fibers, using Dr. Askanas' previously invented "sandwich" technique, and (c) after embedding in plastic a new way for precise plucking (on the basis of light-microscopy selection) of individual fibers for electronmicroscopy or electronmicroscopic-histochemistry. "a" demonstrated good maturation of muscle fibers cultured aneurally, while "b" showed lack of differentiation of them into different histochemical fiber types when cultured aneurally. "c" is a necessary technique to have developed when trying to reproduce in culture the muscle fiber abnormalities present in the original biopsy, since such changes often affect only some fibers and only some parts of fibers. The first EM-histochemistry of cultured human muscle showed generally normal cytochrome-oxidase staining of mitochondrial cristae and limiting membranes in biopsies from "ragged-red muscle fiber" patients, although a few mitochondria contained amorphous unstained material (none had crystals).

Electronmicroscopy (EM) of cytochrome-oxidase-stained mitochondria showed that nearly all of the intramitochondrial crystals of "ragged-red fibers" lack that enzyme stainability. Light-cored dense particles within mitochondria have been found in a case of a myopathy with ragged-red fibers and idiopahtic septal hypertrophy of the heart -- whether those particles reflect viral infection ("mitochondriophages"), calcium abnormality, or other defect is unknown. The new abnormality of "zipper tubules", identified histochemically, has been elucidated by EM.

Autoradiography is being utilized at the electronmicroscopic level to analyse the molecular basis of trophic influence from the lower motor neuron to schwann cells and muscle cells.

Histochemistry -- The systematic approach for application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies we have developed is being emulated in virtually all centers for neuromuscular research throughout the world. This includes basic stains developed (e.g., our modified trichrome), our system of fiber nomenclature, and our new concepts in the analytical approach to neuromuscular pathology (e.g., selective vs. non-selective fiber-type involvement; total-unit vs. partial-unit involvement; complete vs. incomplete defect of neuron-to-muscle influence; fiber-type grouping; fiber-type hypotrophy; and motor-unit hypoplasia). Many investigators and technicians come each year to learn this approach. A number of outside biopsies are sent to us for histochemical processing and/or interpretation. In our basic work, we are extending a technique used in limb muscle of an animal to its extraocular muscle in order to precisely correlate the histochemical and physiological properties of individually studied and marked motor units.

A new nomenclature for human muscle fiber histochemical types, written by us as chairman of the nomenclature committee of the Research Group for Neuromuscular Diseases of the World Federation of Neurology, has been accepted by the committee and published as written. The myofibrillar ATPase (pH 9.4) is

designed as the stain of choice for distinguishing two basic fiber types, called "type I" and "type II" fibers; other reactions, such as for various oxidative enzymes, phosphorylase and lipid content will be useful for identifying various subtypes, and we are now developing a subtyping classification.

Analysis of the rapid events associated with muscle fiber contraction and elasticity, using our specially designed equipment, has added new details on the basic functional properties of muscle of animals, as a model for human muscle function.

Serial No. NDS(I)-62 MN/OC 915(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Histochemistry Applied to the Study of Human Neurologic Disease

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: Husseiny I. Dessouky, M.D.
N. Bojji Reddy, M.D.
Bernard M. Patten, M.D.
David S. Zee, M.D.
David Robinson, M.D.

Cooperating Units: Department of Physiology, Johns Hopkins School of Medicine, Baltimore, Md.

Man Years:

Total:	3.5
Professional:	2.0
Other:	1.5

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle, neurons and peripheral nerves and to see how they are altered in various neuromuscular and other neurologic diseases. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To identify selective or nonselective involvement of a particular histochemical fiber type in the various neuromuscular diseases.

Methods Employed: About 20 histochemical reactions are being done routinely on muscle and neural tissue - they have been outlined in previous reports. New specially developed or adapted reactions include ones for cytochrome oxidase, acid phosphatase, and acetylcholinesterase at the electronmicroscopic level (see our Electronmicroscopy Project).

Numerous interrelated studies between this Project and other Medical Neurology Branch Projects were recorded under those other projects, for example:

a. New techniques of "Open-biopsy Electromyography" for direct correlation of electromyographic and histochemical data from the same fibers, in patients with various neuromuscular disorders - Electrophysiology Project.

b. Histochemistry of human and animal muscle cells and neurons in tissue culture - Tissue Culture Project.

c. Histochemistry of motor neurons and peripheral nerves - ALS Project.

d. Histochemical basis for suggesting that a number of erstwhile myopathic diseases may be neurogenic, e.g., myotonia congenita, paramyotonia congenita, myotonic atrophy ("dystrophy"), type I fiber hypotrophy with or without central nuclei, central core disease, and the type I fiber predominance form of the benign congenital hypotonia syndrome - ALS Project.

e. Histochemistry was the basis of our new approach to defective motor neuron influences on muscle; proposing an analysis of the hypothetical in toto (total-unit) vs. in portio (partial-unit) involvement of the motor unit - ALS Project.

f. Histochemistry integrated with electromyography, was the basis of our new concept of "hypoplastic motor units" in certain neuromuscular disorders, such as benign-congenital-hypotonia-with-type-I-fiber-predominance and central-core disease - ALS Project.

g. Identifying new diseases, such as focal-loss-of-cross-striations and central-cores-with-central-hot-spots - ALS Project.

h. Identifying subtle denervation in patients with primary and secondary hyperparathyroidism - ALS Project.

i. Identifying type-II fiber muscle atrophy associated with a distant neoplasm - ALS Project.

j. Diagnosing amyloid in muscle biopsies of previously undiagnosed patients - ALS Project.

k. Finding rods in nuclei in two cases of adult-onset rod disease, the ultrastructure of which was detailed by electronmicroscopy - Myopathy Project.

l. Correlating end-plate binding of radiolabelled alpha-bungarotoxin - Myasthenia Gravis and Autoradiography Project.

Patient Material: Biopsies obtained for diagnostic purposes were studied histochemically from Medical Neurology Branch patients as well as from consultation patients; patient biopsy material was also submitted by outside physicians and investigators. Some muscle biopsies were grown in tissue culture prior to histochemical study (see our Tissue Culture Project).

Major Findings: In general, the systematic approach for application of a rapid short (5-stain) and long (18-stain) battery of histochemical reactions to fresh-frozen sections of human muscle biopsies we have developed is being emulated in virtually all centers for neuromuscular research throughout the world. This includes basic stains developed (e.g., our modified trichrome), our system of fiber nomenclature, our new concepts in the analytical approach to neuromuscular pathology (e.g., selective vs. non-selective fiber-type involvement; total-unit vs. partial-unit involvement; complete vs. incomplete defect of neuron-to-muscle influence; fiber-type grouping; fiber-type hypotrophy; and motor-unit hypoplasia); and introduction of new, more appropriate descriptive terms (e.g., fiber smallness or largeness). Many investigators and technicians come each year to learn this approach. A number of outside biopsies are sent to us for histochemical processing and/or interpretation.

A new nomenclature for human muscle fiber histochemical types has been accepted and published as a chairman-of-the-nomenclature-committee's original report to the other members of the Research Group for Neuromuscular Diseases of the World Federation of Neurology. The myofibrillar ATPase (pH 9.4) is the stain of choice for distinguishing two basic fiber types while other reactions, such as various oxidative enzymes, are useful for identifying various subtypes, there being at least two in each major type. Already, our system had become the one generally used throughout the world for histochemical fiber typing of human skeletal muscle.

In a biochemical-histochemical correlative study, it was shown by biochemical assay of Ca^{++} activated ATPase activities of isolated myofibrillar, mitochondrial, and sarcoplasmic reticulum (SR) fractions of guinea pig "red" (type-I-fiber) vs. "white" (type-II-fiber) muscle, under exactly the same conditions as employed histochemically (pH 9.4), without or with various degrees of acid pre-incubation, that our previous histochemical impression were confirmed regarding: (a) true reversal of relative amounts of myofibrillar ATPase in type I vs. II fibers, and (b) the additional contribution of the intermyofibrillar membranous components (mitochondria and SR) in creating the greater overall histochemical darkness of type I cf. II fibers. The myofibrillar component, since it contains about 15-fold more protein than the SR and mitochondrial components combined, is likely to be quantitatively the major determinant of type II muscle fiber histochemical coloration in regular (non-preincubated) and acid-preincubated ATPase reactions.

Alkaline-phosphatase-positive muscle fibers, an empiric abnormality in human disease, were produced experimentally.

m. Identifying a "new" (3rd) case of bizarre polysaccharide storage in muscle, heart, and other tissues - Myopathy Project.

n. Identifying the nearly-diagnostic alkaline-phosphatase-positive staining in connective tissue in the dermatomyositis/polymyositis complex - Myopathy Project.

o. Identifying the unique dominantly inherited syndrome of myoclonus epilepsy, ragged-red muscle fibers with bizarre mitochondria, and limb-girdle myopathy - Myopathy Project.

p. Correlating muscle-fiber histochemistry and individual motor-unit function in cat eye muscles, as we have done in the past in cat limb muscles - ALS Project.

Significance to Bio-Medical Research and the Program of the Institute: We have firmly established over the past nine years (see first paragraph above, under "Major Findings") that histochemistry of muscle biopisies is the single most important laboratory evaluation of human neuromuscular disease, and that it is also extremely useful in (a) screening for correlating with electronmicroscopic studies, (b) correlation with electromyographic findings recorded from the same fibers, (c) evaluating growth in tissue culture of human muscle biopsies from patients with various neuromuscular diseases, (d) correlating with other studies on the same biopsy, such as biochemistry, immunology and autoradiography and (e) evaluating animal models. This approach has provided insight into the possibility that certain diseases heretofore called "myopathies" may actually be caused by a form of neuronal abnormality, perhaps related to a defect of neuronal trophic function. This type of analysis (a) helps establish whether the basic pathogenesis and etiology are related to properties reflecting the metabolic "sameness" or the "differentness" of the lower motor neurons or muscle fibers, and (b) directs subsequent investigative studies toward analysing those two groups of properties of muscle and nerve cells. Obviously, knowing whether a certain neuromuscular disease is due to nerve cell or muscle cell abnormality may be critical to understanding its cause and treatment.

Proposed Course of Project: Further correlations of the results from light microscopy with those of electronmicroscopy, tissue culture, physiology, biochemistry, autoradiography and immunology will be made in human neuromuscular diseases and certain normal animal studies. Other animal models of human neuromuscular disorders will be produced and studied.

Honors and Awards: None

Publications:

Burke, R. E., Levine, D. N., Zajac, R. E., Tsairis, P. and Engel, W. K.: Direct correlation of physiological and histochemical characteristics in motor units of cat triceps surae. In Desmedt, J. E. (ed.): New Developments in Electromyography and Clinical Neurophysiology, Basal, Karger, 1973, pp. 23-30.

Campa, J. F. and Engel, W. K.: Histochemistry of motor neurons innervating slow and fast motor units. In Desmedt, J. E. (ed.): New Developments in Electromyography and Clinical Neurophysiology, Basal, Karger, 1973, pp. 178-185.

Engel, W. K.: Histochemical fiber type nomenclature of human sketal muscle. J. Neurol. Sci. 21:507-511, 1974.

Engel, W. K.: Fiber-type nomenclature of human skeletal muscle for histochemical purposes. Neurology 24:344-348, 1974.

Serial No. NDS(I)-62 MN/OC 917(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Barry W. Festoff, M.D.

Other Investigators: N. Bojji Reddy, M.D.
R. Tsukui, M.D.
R. A. Pieter Kark, M.D.
John P. Blass, M.D.
Eugene C. Weinbach, M.D.

Cooperating Units: U.C.L.A., School of Medicine, Los Angeles, Calif.
Physiology and Biochemistry Section, LPD, NIAID

Man Years:

Total:	2.4
Professional:	3.2
Other:	0.7

Project Description:

Objectives: (1) To seek and analyse biochemical abnormalities of neuromuscular and other human neurologic diseases; (2) to evaluate biochemical properties, and possible defects thereof, in specific subcellular components after their isolations and purifications, with special emphasis on characterizing the structural and functional components of the sarcolemmal membrane complex of mammalian (including human) skeletal muscle; (3) to elaborate biochemical differences and similarities between "red" and "white" muscle of animals and between type I (similar to "red") and type II (similar to "white") individual muscle fibers of humans and animals.

Methods Employed: Blood, spinal fluid, muscle biopsy, and cultured fibroblast samples of patients were studied with a variety of techniques, as was rat muscle:

a. Adapted to mammalian muscle have been a number of techniques of isolation and purification of rat and human sarcolemmal membrane.

b. Various membrane-located enzymes studied are mentioned below in each section.

c. As an extension of "a" (above), the mammalian (rat) muscle nicotinic cholinergic receptor has been isolated.

d. ^{125}I-alpha-bungarotoxin was used to identify the nicotinic cholinergic receptor population in biopsies of patients with various neuromuscular diseases.

e. Virtually fiber-type-pure soleus (type I, red) and extensor digitorum longus (EDL) (type II, white) guinea pig muscle was fractionated into sarcoplasmic reticulum (SR) mitochondria, myofibrils, actomyosin, and myosin according to established procedures. These fractions were assayed at pH 9.4 (with calcium as the activating factor) for their ATPase activity without and with EDTA pretreatment at varying pHs (4-10.5) in media exactly corresponding to that used for histochemistry, in order to correlate biochemical subcellular localization with that of histochemistry.

f. Standard isotope techniques for oxidation of succinate, beta-OH-butyrate, palmitate, and pyruvate have been adapted to tissue slices of small biopsy specimens of human muscle.

g. Standard oxygen electrode techniques for the oxidation by pure mitochondrial fractions, isolated from human muscle biopsies and cultured human skin fibroblasts, of succinate, beta-OH-butyrate, palmitate, pyruvate, and NADH.

h. Lactate, pyruvate, and creatine phosphokinase (CPK) of blood and spinal fluid.

Patient Material: Selected Medical Neurology Branch patients with neurological diseases, utilizing portions of specimens obtained for diagnostic purposes.

Major Findings: (1) Characterization of Mammalian Sarcolemma -- Markers for "end-plate" and general membrane function have been identified on density gradient fractions of "crude" sarcolemmal vesicles. These markers have been purified relative to the homogenate of muscle from which they are derived. Acetylcholinesterase (AChE) and acetylcholinergic receptor (as defined by binding of alpha-bungarotoxin, specific for nicotinic receptors), NaKATPase, adenylate cyclase, Ca^{++} binding and uptake, and insulin binding have been assayed in the fractions of discontinuous gradients monitored by electron-microscopy. AChE, NaKATPase and adenylate cyclase preferentially distribute in lighter density fractions while the receptor activity is highest in the mid-to-heavy density bands. This is the first demonstration in mammalian muscle of the molecular distinction between AChE and the ACh-receptor. These results are consistent with the concept that AChE may be superficially bound in the intersynaptic matrix or "fuzz" while the receptor is a component of the true plasma membrane. Our next studies directed at a further molecular

"dissection" of the sarcolemma are in progress. The functional nature of the carbohydrate-rich "fuzz" or basement membrane, the turnover of AChE and receptor in states of denervation and re-innervation are continuing.

(2) Adenyl Cyclase Activity -- This enzyme activity was estimated by isotopic assay. The SL seems to be rich in adenyl cyclase (AC) activity. Most of the enzyme activity was found to be associated with a light density (D_4^{20} 1.0-10) subcomponent of the SL. The SR also possessed AC activity but to a much less extent when compared to SL. Sodium fluoride profoundly stimulated the AC activity of both the SL and SR. Hormones like epinephrine, glucogon, and insulin and cholinergic ligands like carbamylcholine chloride were found to be without effect either along or in the presence of micromolar concentrations of GTP on AC activity of SL and SR. Further characterization of these enzyme properties is under progress.

(3) Production of Specific Antibodies Against Sarcolemmal Components -- In conjunction with above, sarcolemmal membrane fragments have been solubilized with the detergent, Lubral WX. There is release of 60-75% of the membrane protein along with 50-65% of the activities of the membrane-bound components enumerated above. These components have now been separated on DEAE-cellulose and Sepharose 4B columns and specific antibodies to these components have been made in goats. These will be used in our further studies. Antibodies made against alpha-bungarotoxin have been utilized to demonstrate the receptor area in human motor "end-plates" at the light microscopic level; their ultrastructural localization is in progress using the unlabelled-antibody peroxidase anti-peroxidase method. After the pattern in normal human muscle is established, we will look at human diseases in which there is known or suspected abnormality of the receptor at the end plate, as in denervations, central core disease, and myotonic atrophy.

(4) Muscle Membrane Phosphorylation -- Membrane from preps as described in "1" and "2" have been phosphorylated without the addition of an exogenous kinase. Initially we thought this activity represented endogenous kinase and acceptor protein in the same membrane. However, the protein is phosphorylated in as little as 10 secs, its activity is 2-3 times increased with 100 mM Na^+ and is halved by 20 mM K^+. A unique 88,000 MW protein is the apparent phosphoprotein, as determined on SDS-gels. This peak is eliminated by Ca^{++} and a 20,000 MW peptide phosphorylated in its stead. The data are tentatively interpreted as the heavier molecule representing the Na-stimulated, K-inhibited phosphorylated NaKATPase of muscle plasma membranes. The 20,000 MW peptide, phosphorylated in the presence of Ca^{++}, may be similar to the protein species found in the sarcotubular system of skeletal muscle. This may be indicative of a Ca^{++}-transport system inherent in SL, possibly representing the sarcotubular invagination of the SL. Our methods are now sufficiently sensitive that we are able to study the enzyme in membrane preparations of muscle biopsies of our patients with various neuromuscular disorders. The importance of these findings relate to disorders such as myotonic atrophy which have been stated to possess membrane defects involving an endogenous protein kinase, as well as to diseases in which membrane defects are suspected but unproved, such as hypo- and hyperkalemic periodic paralysis.

(5) Biochemical-Histochemical Correlations of Muscle ATPase -- In a biochemical-histochemical correlative study, it was shown by biochemical assay of Ca^{++}-activated ATPase activities of isolated myofibrillar, mitochondrial, and sarcoplasmic reticulum (SR) fractions of guinea pig "red" (type-I-fiber) vs. "white" (type-II-fiber) muscle, under exactly the same conditions as employed histochemically (pH 9.4), without or with various degrees of acid pre-incubation, that our previous histochemical impression were confirmed regarding: (a) true reversal of relative amounts of myofibrillar ATPase in type I vs. II fibers, and (b) the additional contribution of the intermyofibrillar membranous components (mitochondria and SR) in creating the greater overall histochemical darkness of type I cf. II fibers. The myofibrillar component, since it contains about 15-fold more protein than the SR and mitochondrial components combined, is likely to be quantitatively the major determinant of type II muscle fiber histochemical coloration in regular (non-preincubated) and acid-preincubated ATPase reactions.

(6) Oxidative Metabolism in Small Samples of Normal and Diseased Human Muscle -- In slices of human muscle biopsies we last year reported that muscle from more than half the patients with spinocerebellar denervation and 1/3 of cases with idiopathic or familial neuropathy oxidized pyruvate at a rate more than 5 SE below the control mean. We have now demonstrated in the cultured skin fibroblasts of patients from two families with spinocerebellar degeneration (Friedreich's ataxia type) that same biochemical defect. Muscle from 4 patients with myopathies characterized by ragged-red muscle fibers oxidized beta-OH-butyrate more than 3 SE below the control mean. In a family with hereditary myoclonus epilepsy, a concomitant myopathy (with ragged-red fibers) with severe morphologic abnormalities of mitochondria and a biochemical defect of oxidation of beta-OH-butyrate by muscle slices was found. Assuming the same metabolic defect may exist in brain causing the epilepsy, skeletal muscle provides much more accessible tissue for its elucidation.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is important to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity is often neglected, but it is vital to planning future investigative and therapeutic procedures. Both the properties of the general sarcolemmal membrane and the specialized properties of the neuromuscular junctional regions of the sarcolemma hold great promise as a model for excitable membranes, as well as for the elucidation of basic mechanisms in various neuromuscular diseases. Having comparative studies of biochemical differences between type I and type II skeletal muscle fibers will help provide a basis for understanding the pathogenesis of the many neuromuscular disorders preferentially affecting one of these fiber types, as detailed in the Histochemistry Project. The precise localization and characterization of normal biochemical properties to each type of subcellular element of the muscle fiber can serve as the basis for seeking biochemical defects thereof in various human neuromuscular diseases. The spinocerebellar ataxias, usually hereditary, have long been resistant to yielding up their secrets to scientific analysis, but a beginning is now being made in their biochemical analysis.

Proposed Course of Project: Insofar as personnel and space permit, the above techniques will be further perfected and applied to muscle and/or nerve from a variety of human neuromuscular and central nervous system disorders to seek further details of biochemical defects therein. Biochemical differences and similarities of normal human type I and type II fibers will be explored further. Various aspects of the membrane studies to be studied are mentioned in several sections of "Major Findings".

Honors and Awards: None

Publications:

Kark, R. A. P., Blass, J. P. and Engel, W. K.: Oxidative metabolism in small samples of normal and diseased human muscle. II Internat. Cong. on Muscle Diseases, Nov. 22-26, 1971, Perth, Australia, Excerpta Medica Internat. Cong. Series No. 237, p. 63.

Festoff, B. W. and Engel, W. K.: In vitro analysis of the general properties and junctional receptor characteristics of skeletal muscle membranes. Proc. Natl. Acad. Sci. USA, 1974, in press.

Reddy, N. B. and Engel, W. K.: Biochemical characterization of the histochemical "reversed adenosine triphosphatase" in subcellular fractions of muscle fibers. J. Histochem. Cytochem., 1974, in press.

Reddy, N. B., Tsukui, R., Engel, W. K. and Festoff, B. W.: Further in vitro studies of skeletal muscle sarcolemma: Phosphorylation of membrane protein. Trans. Soc. Neurosci., 1974, in press.

Serial No. NDS(I)-63 MN/OC 1034(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators: John N. Whitaker, M.D.
Richard W. Price, M.D.
Barry E. Levin, M.D.
Valerie Askanas, M.D., Ph.D.

Cooperating Units: Veterans Administration Hospital, Memphis, Tn.
I.R.H., New York University Medical Center, New York City

Man Years:

Total:	3.2
Professional:	2.2
Other:	1.0

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical ultrastructural and immunologic abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those parameters. To seek pathogenic mechanisms, using a variety of different techniques including tissue culture and the ones listed above, applied to the patient's body fluids and tissues, especially to the muscle biopsy specimens. To treat myopathic disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological projects of this Branch. Therapeutic trials were conducted with very careful clinical and laboratory examinations to document change of disease state. For comparison with Duchenne muscular dystrophy, non-structural vascular lesions of muscle were produced in animals, the rise of serum "muscle enzymes" followed, and preventative drugs sought.

Patient Material: Diagnostic material from Medical Neurology Branch patients and from outside patients from whom diagnostic muscle biopsies were obtained and sent here for study.

Major Findings: We have previously proposed a new hypothesis, based on muscle histochemical studies, concerning the pathogenesis of Duchenne's progressive pseudohypertrophic muscular dystrophy (DMD). Because the earliest histochemical change is a grouped pattern of degenerating and regenerating muscle fibers, it was hypothesized that the disease is caused by an abnormality of small arterial blood vessels on either a structural or functional basis. That principle was established and the hypothesis shown to be possible by our production of identical early and late muscle lesions after single and repeated embolization of rabbit arteries (structural arterial occlusion). We then reported production of the same early and late Duchenne-like muscle lesions with non-structural, presumably functional, lesions of intramuscular blood vessels by combining a subthreshold vascular factor (aortic ligation) and a subthreshold dose of a vasoactive amine (either serotonin [5-hydroxytryptamine] or norepinephrine), thereby establishing the possibility of a functional abnormality of blood vessels and/or vasoactive substances affecting them being the basis of Duchenne dystrophy (thus like small "strokes" or "transient-ischemic-attacks" of muscle). We then reported that in this model, following each dose of serotonin or norepinephrine in the previously ligated animal there was a striking rise of "muscle enzymes" (CKP, SGOT, SGPT, LDH) in the serum (peak about 12 hours), which returned to normal by 3-5 days, after a single dose, while repeated doses of serotonin caused repeated rises of those enzymes. This demonstrated a further parallel of the model with human DMD and also provided a convenient index with which various therapeutic drugs could be quantitatively evaluated. We have now identified 3 drugs, pretreatment with which has completely blocked the serotonin-provoked muscle damage in this model, and two which prevent the norepinephrine-induced muscle damage, all having typical dose-response curves. One agent has had paradoxical effects, blocking in low doses and enhancing in high doses the muscle-damaging effect of norepinephrine.

The possibility of looking for abnormality of sympathetic innervation of intramuscular blood vessels in Duchenne muscular dystrophy by using fluorescent antibody against dopamine-beta-hydroxylase was thwarted by our finding of great paucity of such positive nerve endings in control muscle (as contrasted with rich innervation of rat vas deferens).

Our attempt to produce a vasculogenic myopathy by a 6-hydroxy-dopamine sympathectomy of newborn mice was unsuccessful -- no abnormality was noted in the muscles.

In myotonic atrophy (myotonic "dystrophy"), several lines of evidence continue to support our hypothesis that muscle involvement in this disease is not myopathic, but due mainly to an altered nerve influence on muscle (and therefore should not be listed in the dystrophy category). We propose that it involves a fractionation of the motor units, not loss of whole motor units as another has proposed.

In myotonia congenita and paramyotonia congenita, also thought by others to be myopathies, we have now postulated a major neurogenic aspect to the pathogenesis on the basis of histochemical evidence, a hypothesis with which other findings are harmonious. Other erstwhile "myopathies" now suspected of being mainly or partly neurogenic are detailed in our Amyotrophic Lateral Sclerosis Project.

The polymyositis/dermatomyosis (PM/DM) disease complex, when beginning over the age of 40, especially in males, has been said to be commonly associated with, and perhaps due to, a small distant cancer. We have not found a cancer in a number of such cases, but have recently started to use a very sensitive serologic test to seek cancer. The childhood form of PM/DM is not associated with cancer. The exact pathogenic mechanism in both groups is unknown. We have now proposed that in the children it might relate mainly to immunoglobulin/complement complexes in intramuscular blood vessels, while in adults it might involve mainly an immunotoxicity of lymphocytes. Our previous immunofluorescent studies support the mechanism proposed for childhood PM/DM, and have now set up the sophisticated methods to evaluate lymphocyte function in the adult form (as well as in the childhood form and in other possible dysimmune neuromuscular diseases). (See our Immunology Project).

The method of treatment we introduced to patients of all ages with the PM/DM complex 6 1/2 years ago, high-single-dose alternate-day prednisone, has now proven in our hands to be the best available, both for children and adults without or with cancer. It has greater therapeutic benefit, fewer side-effects, and is easier to manage. Our experience now includes about 40 carefully studied adults and children, many of them considered therapeutic failures on other regimens. Yet, not all respond, but in 4 very sick patients we have had subsequent remarkable improvement when azathiaprine was judiciously added to the alternate-day prednisone program. Since either type of immunological abnormality proposed in childhood or adult PM/DM could in turn be caused by a viral infection and/or an associated cancer, or the cancer and the "immuno-myopathy" could both be viral-induced, we searched for Australian antigen. We have now reported that none is detectable, either circulating or bound to blood vessels or muscle cells, in any of our PM/DM patients (with CC). Attempts to recover or "rescue" a virus from muscle biopsies of PM/DM patients, in collaboration with 3 different laboratories, have so far failed to reveal a transmissible agent (with NCI, NINDR, PBR, NINDS).

Our original finding of alkaline-phosphatase-positive staining in connective tissue being strongly suggestive of the diagnosis of the polymyositis/dermatomyositis complex, especially of children was given stastical support by our detailed review of 40 PM/DM cases in comparison with 200 cases of other neuromuscular diseases, including 20 cases of Duchenne muscular dystryophy.

In two cases of late-onset rod disease, intranuclear rods, identical histochemically and ultrastructurally to those of the cytoplasm, were found suggesting an origin from (i) a contractile protein common to both nuclear and cytoplasmic sites, or (ii) one "imported" into the nucleus from the cytoplasm. The rod protein is proposed to be actin (possibly and/or tropomyosin) and perhaps alpha-actinin. Whatever the mechanism of rod formation in nuclei, this finding in disease focuses attention on either a neglected or a previously unpostulated mechanism.

One case also had an associated unusual serum paraprotein, IgG/lambda myeloma protein, although its possible relation to the muscle degeneration is not yet known (See our Electron Microscopy Project).

A new clinically delineated "leviated arms syndrome" has been identified in 2 cases and the cause discerned as iatrogenic intramuscular injections into the deltoids; one was very successfully treated by surgical transection of the fibrous bands.

A bizarre polysaccharide material has been found as the storage material in muscle in a rare type of cardio-skeletal myopathy. From the recently obtained autopsy its biochemistry and histochemical distribution in various tissues is being analyzed.

We have previously introduced the term for a characteristic type of muscle fiber abnormality, "ragged red fibers", consisting of fibers with large collections of enlarged mitochondria identified histochemically and confirmed by electronmicroscopy. Ultrastructurally the mitochondria have bizarre whorls of cristae and intra-cristal semi-crystalline inclusions. Ragged-red fibers in limb muscle are often seen in a myopathy accompanying progressive exteral ophthalmoplegia, as we previously reported. This year we added to the group of a a few other diseases known to be associated with "ragged-red fibers" (some with lactic acidosis and some without) one presenting as cardiac involvement with idiopathic septal hypertrophy. Study of the more accessible limb muscle in such patients may help the understanding of cardiac involvement if it is caused by the same biochemical defect. With electronmicroscopic-histochemistry, we have just determined that the intra-cristal semi-crystalline structures of the mitochondria of ragged-red fibers lack cytochrome oxidase (see our Electronmicroscopy Project). Under our Tissue Culture Project we described our studies growing in tissue culture (in collaboration with Dr. Valerie Askanas, New York University Medical Center) muscle fibers from biopsies of patients with ragged-red fibers to determine whether the mitochondrial defects are intrinsic to muscle fibers and detectably reproducible in culture by use of electronmicroscopy-histochemistry and electronmicroscopic-histochemistry of the cultured fibers. New technical procedures have been worked out, with Dr. Askanas, as part of this study.

As described in our Electrophysiology Project, we have specifically criticized the term "myopathic EMG" which, unfortunately in our view, is still widely used even in the other major centers of human neuromuscular research. Our detailed critique has been published.

The principal investigator organized the scientific program of a second 3-day symposium on Neuromuscular Diseases in Houston, March, 1973, under the sponsorship of the Veterans Administration and Baylor Medical School. It was attended by 400 physicians and, as with the first symposium 18 months previously, the principal speakers were recorded on video tape for general distribution.

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electronmicroscopy, biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represents a step toward the overall understanding of this group of disorders. We are now determining whether tissue culture of the abnormal muscle will aid in the diagnosis or understanding of the cause. It is only when the detailed differences of the various forms of myopathy are analyzed that the pathogenesis, etiology, and treatment can be understood. In fact, we are now proposing that some erstwhile "myopathies" (such as myotonic "dystrophy") are the result of abnormal neural influence on muscle. Identification of characteristic histochemical and biochemical defects (such as the inability to utilize long-chain fatty acids and phosphorylase deficiency) in the myopathies has provided leads to further explore the pathogensis is a possible treatment. We are seeking others.

Specific or semi-specific structural changes of certain elements, such as mitochondria, especially when coupled with a metabolic defect, such as lactic acidosis, provide leads toward finding a specific metabolic defect.

Our histologically based hypothesis of ischemia as the cause of Duchenne muscular dystrophy raises for consideration the possible use of drugs to increase the blood flow through perfusion vessels of muscle. The finding of drugs useful in our model for preventing muscle damage further raises the possibility of their use in Duchenne dystrophy patients.

The beneficial response obtained in the therapy of polymyositis/dermatomyositis demonstrates two additional regimens available in the management of such disorders, high-single-dose alternate-day prednisone without or with azathiaprine. Our proposals that childhood and adult forms of polymyositis/dermatomyositis have different pathogenesis might help us to more clearly define the mechanism of each and lead to better, more accurate treatment of each.

Our identification of the iatrogenic cause of the "arm leviation syndrome" can result in its prevention if physicians throughout the country

are aware of the cause, and our demonstration of a treatment will be of benefit to other patients so afflicted.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Honors and Awards: None

Publications:

Engel, W. K.: Duchenne muscular dystrophy: A histologically-based ischemia hypothesis and comparison with experimental ischemia myopathy. In The Striated Muscle, Int. Acad. Pathol. Mono. No. 12, Baltimore, The Williams & Wilkins Co., 1973, pp. 453-472.

Engel, W. K.: "Ragged-red fibers" in opthalmoplegia syndromes and their differential diagnosis. II Int. Cong. on Muscle Diseases,Nov. 22-26, 1971, Perth Western Australia, Excerpta Medica Int. Cong. Series No. 237, p. 28.

Levin, B. E. and Engel, W. K.: Arm levitation syndrome from iatrogenic muscle fibrosis. Neurology 23:439, 1973.

Whitaker, J. N. and Engel, W. K.: Mechanisms of muscle injury in idiopathic inflammatory myopathy. New Engl. J. Med. 289:107-108, 1973.

Whitaker, J. N., Holland, P. V., Alter, H. J. and Engel, W. K.: Idiopathic inflammatory myopathy. Arch. Neurol. 28:410-411, 1973.

Engel, W. K. and Borenstein, A.: Histochemical evidence raising the question of a neurogenic aspect in myotonia congenita (MC) and paramyotonia congenita (PM). J. Neuropathol. Exp. Neurol. 33:192, 1974.

Serial No. NDS(I)-63 MN/OC 1037(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Tissue Culture Applied to the Study of Human Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Valerie Askanas, M.D., Ph.D.
Mark S. Horwich, M.D.

Other Investigators: None

Cooperating Unit: Muscle Research Laboratory, Institute for Physical Medicine and Rehabilitation, N.Y. University School of Medicine, N.Y., N.Y.

Man Years:

Total:	2.4
Professional:	1.4
Other:	1.0

Project Description:

Objectives: To study the growth characteristics, histochemical and biochemical reactivities, immunologic properties, and electronmicroscopy and autoradiographic details of human and animal skeletal muscle and animal neurons grown in tissue culture. To perpetuate abnormalities of human cells (muscle, schwann cells, fibroblasts) in culture, or to induce those abnormalities in cultured normal human or animal tissues. To compare the growth and histochemical characteristics of "red" vs. "white" muscle in culture. To study interactions between lower motor neurons and muscle fibers in culture. To seek growth-inhibitory ("toxic") factors in body fluids of our patients, using cultured neurons or muscle cells as test objects. The tissue is from either normal or abnormal biopsied adult or child human tissue, especially muscle, or from normal chick, mouse or rat embryos.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human cells. Applied to the cultured cells are other techniques being used in the Branch, e.g., histochemistry, electronmicroscopy, and immunology. Soon we will be

using autoradiography. For the past two years we have been concentrating the efforts of our tissue culture laboratory on neuronal cultures, while the cultures of human muscle from our patients has been done in collaboration with Dr. Valerie Askanas (N.Y. University) (v.i. for details).

Patient Material: A portion of the open muscle biopsy from certain adult and child patients with neuromuscular disease is available for culture purposes. Muscle obtained by needle biopsy, used for repeat specimens or samples from extremely fragile infants, has been cultured from our patients.

Major Findings: Although tissue culture techniques have been used extensively in studying the development of muscle from animal embryos, human skeletal muscle has been difficult to culture. We previously developed and published a system in which we achieve reproducible growth in Maximow slides from 80% of explants of muscle biopsies of children and adults.

Now our human muscle biopsies are able to be grown more conveniently and even better quantitatively and qualitatively in plastic petri dishes in the laboratory of Dr. Askanas. We have worked out the methods of preparing the specimens in our tissue culture laboratory and sending them to Dr. Askanas in New York. To the cultured fibers she has applied the histochemical reactions of NADH-TR, phosphorylase, modified trichrome, hematoxylin-eosin, and three different ATPase reactions to both longitudinal whole-mounts and cryostat cross-sections, the latter for the first time to human cultured muscle, using the new "sandwich technique" she developed for cultured animal muscle. This study has shown that the aneurally cultured human fibers do not on their own differentiate into distinct fiber types (presumably motor neuron trophic influence, different patterns of activity, or both are needed for fiber type differentiation). For electron-microscopy (see our EM Project), the cultures are histochemical-EM prestained, embedded, cell-selected, and blocked in New York, with the sectioning and electron-microscopy then being done in our EM laboratory. Although the logistics of this system have been well worked out, more precise informational feed-back control will be achieved when the sectioning can be done there and the sections brought here by Dr. Askanas for her study by EM with us -- and this will ensure even better correlation of EM and histochemical data from the cultured fibers with those of the original biopsy specimen of that patient as studied in our laboratories here.

Because abnormalities in human disease often affect only an occasional fiber or part of a fiber we have developed, with Dr. Askanas, a new technique for embedding, prestaining the whole culture for EM-histochemistry and for cell identification, and quickly and precisely plucking (on the basis of selection by light-microscopy) those individual muscle fibers of greatest potential EM interest. Details of all these technical advances have been prepared for publication.

A major focus of the culturing of human muscle fibers has been to seek whether biopsies from patients with "ragged-red" and "rugged-red" fibers (ones with abnormal bizarre mitochondria, see our EM Project) reproduce that change when growing in vitro, free of other body influences. To date, we have

demonstrated good growth of such biopsies in culture and have obtained fibers with mitochondria nicely stained by the EM cytochrome oxidase method and have found suggestive abnormalities of mitochondria, but no distinct intramitochonrial semi-crystalline structures or light-cored dense particles have yet been found.

We are also attempting, with Dr. Askanas, to produce ragged/rugged-red fiber kind of mitochondrial abnormalities by applying various drugs to tissue cultured normal human and animal muscle.

Serum from 70% of amyotrophic lateral sclerosis (ALS) patients has been reported by others to be "toxic" to three-day-old mouse spinal cord motor neurons maintained in tissue culture. We tried to confirm those results, using both three-day-old and embryonic mouse cords, and have now reported that we failed to find any difference between 13 ALS sera and 13 normal and other-disease control sera. The definitive analysis involved 161 explants, 71 treated with control sera and 90 with ALS sera.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical properties of normal muscle and its growth characteristics in vitro, the studies of spontaneous or induced pathologic changes in human muscle in vitro will be used to seek disturbed growth patterns, and morphologic and biochemical abnormalities, in order to glean clues to their pathogenesis and possible treatment. More particularly, ways of improving deficient growth will be sought. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient with muscle disease in respect to promoting growth of abnormal muscle or retarding its degeneration. Study of motor neurons grown in vitro is part of a multilateral approach to diseases which preferentially involve human motor neurons, such as amyotrophic lateral sclerosis (ALS). Failure to confirm a toxic factor in ALS sera means that different tissue-culture technical approaches must be made or other possibilities for pathogenesis must now be considered.

Proposed Course of Project: Studies of normal and pathologic human muscle in tissue culture will be extended and new variations applied.

Honors and Awards: None

Publications: Horwich, M.S., Engel, W.K. and Chauvin, P.B.: Amyotrophic lateral sclerosis sera applied to cultured motor neurons. Arch. Neurol. 30:332-333, 1974.

Askanas, V. and Engel, W. K.: A new program for investigating adult human skeletal muscle grown aneurally in tissue culture. Neurology, 1974, in press.

Serial No. NDS(I)-63 MN/OC 1039(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases, Including Peripheral Neuropathies and Spinocerebellar Ataxias

Previous Serial Number: Same

Principal Investigators:
W. King Engel, M.D.
Mark S. Horwich, M.D.
Bernard M. Patten, M.D.
John R. Warmolts, M.D.

Other Investigators:
John N. Whitaker, M.D.
Peter K. Re', M.D.
Jay H. Robbins, M.D.
Larry E. Mallette, M.D.
Gerald D. Aurbach, M.D.
Justino F. Campa, M.D.
Robert J. Schwartzman, M.D.
R. A. Pieter Kark, M.D.
John P. Blass, M.D.
Eugene C. Weinbach, M.D.
Randall J. Lewis, M.D.
John W. Griffin, M.D.
Richard W. Price, M.D.

Cooperating Units:
Dermatology Branch, NCI
Metabolic Diseases Branch, NIAMDD
Surgery Branch, NCI
Laboratory of Parasitic Diseases, NIAID
Laboratory of Experimental Pathology, NIAMDD
Laboratory of Oral Medicine, NIDR
Depts. of Neurology & Biochemistry, U.C.L.A., School of Medicine, Los Angeles
Dept. of Neurology, University of Miami, School of Medicine, Miami
Dept. of Neurology, University of Virginia School of Medicine, Charlottesville
Dept. of Neurology, V.A. Hospital, Memphis

Man Years:

Total:	2.8
Professional:	2.6
Other:	0.2

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific or symptomatic therapeutic response; (d) new methods of analysing the abnormalities; and (e) animal models of the human pathophysiologic states.

Methods Employed: The techniques of histochemistry, biochemistry, electronmicroscopy, immunology, tissue culture, electromyography and autoradiography were as detailed in other project reports on those methods. Additional methods were as follows. Double-blind, single-blind and non-blinded therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us for quantitating isometric muscle tension. Analysis of motor areas of brain and spinal cord by histochemistry and electronmicroscopy. Biochemical assays of several parameters of oxidative enzyme metabolism (with U.C.L.A.).

Patient Material: Medical Neurology Branch patients and neurology consultation patients from the various services of the Clinical Center, who had diagnostic muscle biopsy and other diagnostic procedures.

Major Findings:

MOTOR NEURON DISORDERS

In ALS, different approaches continue to fail to disclose the cause. Another investigator has reported that sera from 70% of ALS patients but none (sic!) of numerous disease-controls was selectively toxic to motor neurons of 3-day mouse embryos explanted in tissue culture. However, in a blinded, careful study of 13 ALS and 13 normal and disease control sera, involving a total of 161 explants we have reported that we failed to find any difference between normal and ALS sera -- neither was especially toxic to the cultured neurons (see our Tissue Culture Project).

In 16 patients with primary hyperparathyrodism we have reported that there is neurologic abnormality in nearly every case, the most common involvement being proximal muscle weakness, attributed on the basis of EMG and histochemical studies to abnormality of lower motor neurons. There is also sometimes bulbar involvement, tongue tremors, and brisk reflexes, the total picture, when extreme, giving an ALS-like syndrome, which reverts to or toward normal

following surgical treatment of the hyperparathyroidism (with NIAMDD). The only CSF abnormality detected was a low phosphate in about half the cases.

In 8 patients with secondary hyperparathyroidism we have found proximal muscle weakness and wasting to be a common abnormality. Histochemically it is characterized especially by type II fiber atrophy, but because some atrophic fibers are esterase-positive or NADH-TR-positive it is concluded that the muscle involvement is probably neurogenic.

"Benign focal amyotrophy" has been named and reported as a newly clarified disorder (? syndrome) simulating the lower motor neuron aspects of ALS at its onset but of spontaneously limited progression.

We reported that of 18 histochemical reactions done on the cat lumbar cord, in an attempt to learn metabolic particularities of alpha-motoneurons in the anterior horn of the spinal cord (the ones preferentially involved in ALS), only two distinguished them from the others (gamma efferents, interneurons, and Renshaw neurons). Alpha-motoneurons of both fast-twitch and slow-twitch motor units were rich in phosphorylase and poor in succinate dehydrogenase, in contrast to the opposite characteristics of the others. Since human motor neurons, too, are rich in phosphorylase, one theoretical possibility is that ALS might be related to a primary or secondary abnormality of glycogen metabolism (it is known that lower motor neurons are involved in the glycogenesis of infantile acid maltase deficiency).

Previously we have correlated muscle fiber histochemistry with functional properties of individual motor units in cat limb muscle -- now we are attempting the same correlations in cat extraocular muscle.

New hypotheses on the pathogenesis of some neuromuscular disorders that we reported previously have continued to be our "first choice" explanation for those diseases -- specifically that some erstwhile "myopathic" disorders are actually caused by a lower motor neuron abnormality. It is proposed that in spite of a "myopathic" (which we consider often to be a misnomer) EMG (a "BSAP" EMG by our designation) certain disorders are not myopathic but rather neurogenic (our new approach to EMG studies is described in the Electromyography and Clinical Neurophysiology Project). As part of this formulation, details have been proposed regarding how a motor neuron might be defective in a variety of qualitative or quantitative ways with a variety of time courses toward death. Each of these could give a different clinical disorder. Diseases newly considered to be probably neurogenic are: some forms of type II muscle fiber atrophy (cachectic and disuse atrophy, cortico-steroid atrophy ["myopathy"]), myasthenia gravis, facilitating myasthenic syndrome, myotonic atrophy (myotonic "dystrophy"), myotonia congenita and paramyotonia congenita, type I fiber hypotrophy with central nuclei, central core disease, benign congenital hypotonia (with type I fiber predominance), and facioscapulohumeral syndrome (some cases). Possibly neurogenic (perhaps

only in a small part) are thyrotoxic atrophy ("myopathy"), some cases of facioscapulohumeral disease, focal loss of cross-striation, and late-onset and congenital rod disease. From such proposals a new classification of neuromuscular disorders has been reported.

Further evidence was adduced by ourselves and also by others to support our original hypothesis that central core disease is more likely to be an abnormality of the neural influence on muscle than a myopathic disorder as originally proposed by others. An updated full review of central core disease has been written in a book chapter; also covered were full details of the entity we originally described as "focal loss of cross-striations", one in which we have postulated a lower motor neuron defect in spite of the BSAP EMG.

On the basis of histochemistry and electronmicroscopy, a new disorder was reported; a new form of central core disease in which there is a "hot spot" of enzymatic activity and lipid droplets in the center of the core.

A new form of the benign congenital hypotonia syndrome was described, consisting of marked type I muscle fiber predominance -- this was proposed to be caused by an in utero time-limited neuropathy affecting the type I motor neurons resulting in "motor unit hypoplasia" of type I and possibly some type II units (most of the type II units are proposed to be either seriously affected or absent to account for the severe type II fiber paucity).

A new clinico-pathologic syndrome of type I muscle fiber smallness without central nuclei has been found in a child with progressive weakness; it was proposed to be of neural origin.

The new concept of congenitally "hypoplastic" motor units was introduced to explain diseases of congenital non- (or only slowly) progressive muscle weakness associated with type I muscle fiber predominance (probably actually on the basis of type II fiber paucity) and a BSAP EMG pattern. Since the type I motor units fire earliest in EMG recording of voluntary units and in the diseases considered the units are measured as BSAP, one must harmonize that finding with the apparent structural normality of the I fibers which are present in moderate or marked predominance. Our concept is that existing type I units are "hypoplastic" (fewer than normal number of fibers per motor unit) and that type II units are, to account for paucity of II muscle fibers, either non-existent or severely hypoplastic -- and that these changes are caused by an <u>in utero</u> time-limited disorder of the lower motor neurons.

The generalized muscle cachectic atrophy and weakness, which is a common and often the most crippling manifestation of early focal cancer as well as of hypercorticosteroidism (Cushing's syndrome and iatrogenic) and of disuse, histochemically is selective atrophy of type II muscle fibers. We have previously reported a new hypothesis to the effect that this type II atrophy, at least in some cases, may be a pan-neuropathy of all lower motor neurons to which the type II muscle fibers are more susceptible, and might not be a myopathy as is usually stated. That proposal was supported by our report that the ultrastructural aspects of type II atrophy are qualitatively indistinguishable from typical denervation atrophy, but milder in degree. We have shown

that usually there is no alteration of the EMG pattern in type II atrophy, either early or when the patient is severely wasted and weak, probably because the early-firing motor units usually measured during voluntary effort are the type I units (see our Electromyography and Clinical Neurophysiology Project). We have found (with NCI) in patients with non-neurologic, localized (non-metastatic) cancer, before there is clinical evidence of anorexia, weight loss, or decreased daily activity, significant type II fiber atrophy by muscle biopsy. The mechanism of the atrophy in such early-cancer patients is unknown, but at least some of it seems to be on the basis of defective lower motor neuron influence on muscle. This is especially likely to be so in biopsies with II-fiber atrophy in which there are scattered small angular fibers excessively dark with NADH-TR and non-specific esterase stainings (i.e., ones like those in ordinary denervation). We plan to turn our attention to ways to prevent or treat type II atrophy, which would significantly benefit cancer patients even if the basic neoplasm could not be excised.

We have begun using autoradiography with alpha-bungarotoxin to identify post-junctional receptor substance in human muscle fibers and to seek alterations of its spatial pattern in various neuromuscular diseases, to learn more about possible alterations of lower motor neuron influence on muscle (see our Autoradiography Project).

The trophic influence of the lower motor neuron on schwann cells and muscle cells is also being studied by autoradiography combined with electron-microscopy in animal models (see our Autoradiography Project). This should tell us about normal trophic mechanisms and help us consider derangements thereof in various neuromuscular diseases and peripheral neuropathies.

We reported more details of our correlation of histochemistry of muscle fibers with their electrophysiologic properties in individually neuron-soma-stimulated muscle-units of individual motor-units in cat gastrocnemius (see our Histochemistry Project). We are now attempting to do the same thing with motor units of the cat extraocular muscles (see our Histochemistry Project).

PERIPHERAL NEUROPATHIES

Our treatment of a number of cases of idiopathic peripheral neuropathy with long-term high-single-dose alternate-day prednisone (LT-HSDAD-Pred) continues to be very successful and necessary, e.g., for as long as 9 years in an adult and 6 1/2 years in a child. Correlative studies indicate that the patients most likely to respond are dysschwannian in type (slow nerve conduction times), relapsing, and with elevated CSF protein; but even some patients with a dysneuronal type of peripheral neuropathy, non-relapsing, or normal CSF have responded (see our Myopathy Project for details of the LT-HSDAD program).

Immunoglobulin survey of a large group of cases of peripheral neuropathy (PN) currently considered idiopathic was reported to have disclosed elevation of serum IgM in some, raising the possibility of a previously unsuspected immunologic pathogenesis in them.

A new disorder was reported in 7 patients, family sensory PN with elevated serum IgA, shown to be due to IgA overproduction in the small bowel tissue -- on this basis a new hypothesis was introduced, one of possible familial susceptibility to infection in the gut tissue (in which there are excessive eosinophils) that causes through, or parallel to, the IgA overproduction a metabolic abnormality affecting sensory nerves.

Primary amyloidosis has been described in a number of patients with idiopathic sensory neuropathy, studied without a diagnosis being made. Also found was the universal presence of immunoglobulin fragments (e.g., kappa or lambda light chains) in the serum and/or urine and the frequent relationship to myeloma. Treatment with alkeran has not been dramatically successful. We have also contributed to the studies of these patients by Dr. Geroge Glenner (NIAMDD) analysing the molecular defect of amyloid protein formation, and we are pursuing the exact pathogenesis of the neuropathy with a view toward therapeutic intercession.

Regarding herpes simplex virus infection of trigeminal and dorsal root sensory ganglia, the basis mechanisms of latent infection and re-activation of reaction following nerve injury have been partially clarified in experimental animals (with NIDR).

The peripheral neuropathy of xeroderma pigmentosa was identified and its possible etiology speculated upon: it was hypothesized that the previously known defective DNA-repair mechanism might cause a shortened life in non-mitotic (neuronal) cells (enhanced neurologic "aging") because of inability to properly repair ordinarily banal neuronal DNA injuries (with NCI).

Acquired pupillary dysautonomia (light-hyposensitive, tonic, mecolyl-hyper-sensitive) has been identified and shown to be a usually-overlooked but not infrequent (11 of 16 patients) accompaniment of idiopathic non-familial neuropathy, especially when there is a major sensory component (with NEI).

The mechanism (a) of axoplasmic flow in motor neuron axons, and (b) aspects of trophic influence of the lower motor neuron on schwann cells and muscle fibers, are being analysed by autoradiography and EM-autoradiography, and techniques are being developed to analyse these parameters in human nerve biopsies (see our Autoradiography and Electronmicroscopy Projects).

SPINOCEREBELLAR ATAXIAS

In only a very few of the various types of hereditary spinocerebellar degeneration is a metabolic abnormality known. Previously we have reported pyruvate decarboxylase deficiency in a patient with intermittent cerebellar ataxia (who also had excessive lipid droplets in his skeletal muscle fibers). We also have reported previously, using a system we developed for applying radioisotopic biochemical assays to small samples of biopsied human muscle, that the muscle biopsy slices from more than half the patients with spinocerebellar degeneration (as well as 1/3 of cases with idiopathic or familial neuropathy) oxidized pyruvate at a rate more than 6 SE below the control mean;

the oxidation rates were not related to the severity of any neuropathic changes in the muscle. Most recently, in two unrelated patients with Friedreich's ataxia, in whom we had previously shown impaired oxidation of pyruvate by their muscle slices, cultured skin fibroblasts have been shown to oxidase 1-^{14}C-pyruvate and 2-^{14}C-pyruvate at less than half the rate of (but U-^{14}C-glutamate at rates comparable to) those found in normal fibroblasts (see our Biochemistry Project) -- this study is being prepared for publication.

In an investigation of oculomotor dysfunction in spinocerebellar ataxias, eye movements recorded with both an infra-red pupil tracker and electro-oculography allow evaluation of the three major oculomotor subsystems, saccadic (refixations), pursuit (smooth tracking), and vestibular (nystagmus). Thus far, two groups of patients have been discerned, one in which saccadic eye movements are abnormal and patients use a "voluntary" smooth pursuit mode to re-fixate, and a second in which the major defects are in both holding fixation and the pursuit system.

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of at least some patients with yet-idiopathic forms of ALS, peripheral neuropathies, the spinocerebellar ataxias, and other diseases of the lower motor neuron. Identifying known and currently treatable underlying diseases (e.g., primary or secondary hyperparathyroidism) in some of these clinical syndromes provides such patients with cures and gives possible leads to understanding the more numerous yet-idiopathic cases. New aspects of the beneficial use of prednisone in some cases of apparently subtly dysimmune chronic neuropathy indicate additional patients should be tried on the drug on a prolonged program, every-other-day, even if they have been severely paralysed months or longer.

If our proposals are correct that some erstwhile myopathic disorders are actually caused by abnormalities of the lower motor neuron, then all biochemical searches for the basic abnormality that are focused on the muscle represent explorations in the wrong forest -- we must direct our attention to the motor neuron rather than the muscle.

Fuller understanding of the mechanism by which a focal cancer produces generalized muscle weakness and wasting could lead to a means of treating that often-most-disabling aspect of cancer even when the basic neoplasm itself cannot be treated -- such would be of major benefit to a great number of cancer patients.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders. To further explore the interactions of motor neuron and muscle, and disorders thereof. To undertake additional therapeutic trials. We are intrigued with the possibility of treating the cachectic muscle wasting aspects of cancer patients.

Honors and Awards: None

Publications:

Campa, J. F. and Engel, W. K.: Histochemistry of motor neurons innervating slow and fast motor units. In Desmedt, J. E. (ed.): New Developments in Electromyography and Clinical Neurophysiology, Basel, Karger, 1973, pp. 178-185.

Engel, W. K.: Motor neuron disorders. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Central core disease and focal loss of cross-striations. In Shy, G. M., Goldensohn E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Rod (nemaline) disease. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Muscle fiber hypotrophies. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease. In press.

Engel, W. K.: Basic aspects of lower motor neuron abnormalities. In Shy, G. M., Goldensohn, E. S., and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Diseases. In press.

Engel, W. K. and Warmolts, J. R.: The motor unit: Diseases affecting it in toto or in portio. In Desmedt, J. E. (ed.): New Developments in Electromyography and Clinical Neurophysiology, Basel, Karger, 1973, pp. 141-177.

Patten, B. M., Bilezikian, J. P., Mallette, L. E. Prince, A., Engel, W. K. and Aurbach, G. D.: Neuromuscular disease in primary hyperparathyroidism. Ann. Intern. Med. 80:182-193, 1974.

Aurbach, G. D., Mallette, L. E., Patten, B. M., Heath, D. A., Doppman, M. D. and Bilezikian, J. P.: Hyperparathyroidism: Recent studies. Ann. Intern. Med: 79:566-581, 1973.

Mallette, L. E., Engel, W. K. and Patten, B. M.: Neuromuscular disease in secondary hyperparathyroidism and familial hypophosphatemia. Neurology, 1974, in press.

Robbins, J. H., Kraemer, K. H., Lutzner, M. A., Festoff, B. W. and Coon, H. G.: Xeroderma pigmentosum. Ann. Intern. Med 80:221-248, 1974.

Walz, M. A., Price, R. W., Notkins, A. L.: Latent ganglionic infection with herpes simplex virus. Types 1 and 2: Viral reactivation in vivo after neurectomy. Science 184:1185-1187, 1974.

Serial No. NDS(I)-69 MN/OC 1189(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Barry E. Levin, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To define more clearly and to treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemma, T-system, sarcoplasmic reticulum, myofibrillar complex (i.e., the total excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding abnormalities of pertinent metabolic pathways and methods of treatment.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron microscopy, and biochemical assays of tissue are delineated in our other projects. Provocation loading tests and therapeutic trials to raise or lower potassium or sodium were used. Acetazolamide was administered as a prophylactic agent for hypokalemic periodic paralysis.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated

examples of periodic paralysis with potassium disturbances; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonic congenita. (Patients with myasthenia gravis are part of another Medical Neurology Branch project by that name.)

Major Findings: A new use for acetazolamide (Diamox), a carbonic anhydrase inhibitor, was introduced by us 8 years ago -- it is now widely accepted as the best prophylatic agent available in hypokalemic periodic paralysis (LoKPP). Our on-going follow-up discloses that it has thus far provided moderate to striking benefit for 21 of our 23 patients, two for over 8 years. It has also improved the moderate or severe persistent proximal weakness between attacks that afflicted 17 patients on convential therapy. The mechanisms of acetazolamide action in this disease remains unknown, since muscle itself has no carbonic anhydrase. One patient has had a renal stone, of uncertain relation to the acetazolamide. All patients are being monitored for renal stones by x-rays twice yearly.

Paramyotonia congenita has chemical features of both hyperkalemic periodic paralysis (HiKPP) and also myotonia congenita, viz. episodic weakness associated with a rising serum K^+ and widespread myotonia respectively. Although both LoKPP and HiKPP traditionally have been thought of as primary diseases of muscle (i.e., myopathies), we have recently reported that there is in both paramyotonia congenita and myotonia congenita histochemical evidence which we interpret as definite denervation in the muscle. Thus we have offered a new hypothesis that the muscle weakness and myotonia of paramyotonia congenita and myotonia congenita might be on the basis of muscle fiber abnormality induced by abnormality of the lower motor neuron (LMN) two other alternatives are possible: The disorders are myopathies in which there is an aspect of non-receptiveness to LMN influences and/or that both LMN and muscle are involved concomitantly).

Significance to Bio-Medical Research and the Program of the Institute: Acetazolamide has proven to be the most effective single prophylactic agent available in the treatment of hypokalemic periodic paralysis. It provides new avenues for investigation of the basic pathophysiology in this disease. If the hypothesis of a neural basis of paramyotonia congenita and myotonia congenita is correct, that would change the direction of research into their cause and treatment.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even better therapeutic agents. To seek further evidence for or against the hypothesized neural basis or paramyotonia congenita and myotonia congenita.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 MN/OC 1190(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Myasthenia Gravis (MG)

Previous Investigators: W. King Engel, M.D.
Bernard M. Patten, M.D.
Michael L. Swerdlow, M.D.

Other Investigators: John N. Whitaker, M.D.
Charles L. McIntosh, M.D.
John L. Trotter, M.D.
Jay D. Cook, M.D.

Cooperating Units: Department of Neurology, V.A. Hospital, Memphis, Tenn.
Surgery Branch, IR, NHLI

Man Years:

Total:	2.6
Professional:	1.6
Other:	1.0

Project Description:

Objectives: To apply clinical, immunologic, histochemical, pharmacologic, electrophysiologic and electronmicroscopic techniques to investigate the etiology and pathogenesis of myasthenia gravis. To seek new or improved methods of treatment.

Methods Employed: Clinical studies, including edrophonium, prostigmine, curare and lactate challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases. Competence of neuromuscular transmission was studied neurophysiologically in patients with myasthenia gravis and other neuromuscular disorders. Experience was extended and modified with a new treatment program introduced by us, consisting of long-term high-single-dose (100 mg for the average adult) alternate-day prednisone with adjuncts of supplemental potassium, antacids, and low-sodium, low-carbohydrate and high-protein diet. A rat nerve-muscle preparation was used to seek

(a) possible adverse interaction between anticholinesterase and anticholinesterase drugs; and (b) an inhibitory factor released by MG thymus tissue during 24-48 hour culture in vitro. Immunologic, autoradiographic, histochemical and electronmicroscopic methods are listed under those projects.

Patient Material: Myasthenia gravis patients participated in the therapeutic trials. Sera, muscle, thymus and other tissue were obtained during diagnositc or therapeutic procedures from Medical Neurology Branch patients.

Major Findings: The new treatment that four years ago we introduced for adult myasthenia gravis (MG), namely high-single-dose alternate-day prednisone, has continued to give excellent results in 20 of 22 adult patients we have thus far treated, proving to be much more effective therapy than anticholinesterase drugs. It seems especially effective in older males, but both sexes of all age groups have responded. Side-effects of our long-term treatment have been present but relatively minimal, in the form of osteoporosis of some older females, subclinical or clinical cataracts, and, in a rare patient, aggrevation of pre-existing diabetes mellitus or hypertension. Gastrointentinal ulcers or bleeding, even in patients with a previous ulcer history, have not occurred. Since it was potentially too hazardous to take critically ill myasthenia gravis patients off all other anti-myasthenia drugs and let them be nursed on a respirator for 1-2 months while they would have been on placebo, other evidence that this treatment is responsible for the benefit must be weighed. Favoring prednisone as the beneficial factor are: (a) the patients had become unresponsive to all their other treatments prior to being started on prednisone; (b) the upward inflection in their strength curve always began a few days to weeks after prednisone was started and was more-or-less continuously upward thereafter; (c) they did not improve when their anticholinesterases were stopped prior to prednisone; (d) a distinct benefit-worsening cycle related to on-day vs. off-day prednisone was demonstrated, especially early in the course of treatment, evident by clinical examination, respiratory function measurements, and EMG-measured synaptic efficiency; and (e) in some patients the myasthenic state returned as the prednisone dosage was lowered to a certain level and improved again with raising the dosage. This treatment program is still in a long-term investigation phase.

A male with juvenile myasthenia gravis has now been maintained at about 95% improvement for 7 years on corticosteroids, the last 4 1/2 years on the program new for this disease, high-single-dose alternate-day prednisone. A second juvenile myasthenic male has also shown beneficial response for 52 months, and a third male for 30 months. This is in contrast to the short-term response from the short courses of ACTH previously given for juvenile (or adult) myasthenia gravis. As yet, no juvenile has failed to respond to our program.

Some of our clinical impressions have raised the question of an adverse interaction between corticosteroid (prednisone) and anticholinesterase (pyridostigmine or neostigmine). Accordingly, such an effect was sought and found with a rat nerve-muscle test preparation, and reported. Steroid (hydrocortisone) abolished twitch-augmenting effects of pyridostigmine and neostigmine, and once steroid had abolished the anticholinesterase effect, equivalent doses of the anticholinesterase were less effective than before steroid had been given. In water-bath experiments, using frog rectus muscle it was shown that prednisolone and hydrocortisone in concentrations of 0.4 micromole/ml can block contractions induced by acetylcholine; steroid hormones had 1/10th the blocking activity as did d-tubocurarine in this preparation. If these findings relate to the situation in the myasthenic patient they explain why some myasthenics taking corticosteroids and anticholinesterases might, during some periods, do worse than those taking steroid alone. However, a detailed look to determine such possible adverse interactions in MG patients taking both drugs must now be done. Recently, we have cautiously combined the two drugs, using small doses of one or the other, in some MG patients without evident adverse interaction.

The complete hypothesis utilizing data from many types of investigative studies, that we reported previously, to the effect that myasthenia gravis might be a disorder of the lower motoneuron, continues to be our explanation of choice for the pathogenesis of MG. Equally possible, hypothetically, are two types of lower motor neuron defect: (a) the soma fails to provide a qualitatively and/or quantitatively adequate axoplasmic flow to nourish the axon tips, or (b) the axonal tips themselves are selectively involved. This motoneuron defect is proposed as being secondary to an abnormality of thymus and related tissues. Thus the so-far discovered "immunologic" alterations in MG are, by this hypothesis, considered parallel and potentially informative phenomena but not pathogenic for the muscle weakness. The actual link between the abnormal thymus and the neuromuscular weakness is still being sought, v.i. The motoneuron abnormality is viewed as initially causing a greater defect of excitation factor (acetylcholine) and later a diminution of "trophic factor" too.

Our staff presented an N.I.H. Clinical Center Conference on myasthenia gravis, summarizing the current status and our own studies regarding clinical, pharmacological, junctional-physiological, treatment and nursing aspects. This conference is being published, and we have also put it on video-tape for general distribution.

Lactate infused cautiously intravenously was shown to provoke the patient's characteristic weakness in myasthenia gravis patients. Our initial impression is that it is a useful test for diagnosing MG and safer than the widely used curare test.

We had previously shown that the serum antimuscle-antibody characteristic of MG is an immunoglobulin G and a true antibody. Using electronmicroscopy and immunolocalization with peroxidase-labelled reagents, we have now reported that that antibody localizes to the sarcoplasmic reticulum but exclusively at

the I-band level. This is the first demonstration of a structural-chemical difference between the reticulum at the I vs. A bands. (See our Electron-microscopy Project.)

To study the specific acetylcholine-receptor at the neuromuscular junction, alpha-bungarotoxin was purified, and either radioisotopically-labelled or used to generate specific antibodies against it. We have auto-radiographically/histochemically demonstrated the highly specific binding of our preparation to the neuromuscular junction, and will be seeking abnormalities thereof in myasthenia gravis, as well as in other neuromuscular diseases in which we have hypothesized defects of neuron-to-muscle trophic influences (see also our Biochemistry Project).

The basic molecular aspects of the acetylcholine-receptor substance and the specific acetylcholinesterase, both substances highly concentrated at the neuromuscular junction, have been analysed for the first time in mammalian muscle by us. They were shown to be different molecules in distinctly different sarcolemmal membrane fractions, the former probably in the plasma-lemma and the latter possibly in the outer "fuzz" (see our Biochemistry Project). We now expect to be able to perform the same assays, for the first time, in human muscle.

A serum antibody in MG patients specifically against neuronal nuclei but not against nuclei of other cells (except spermatogonia) was reported by another group. We have now reported that our studies fail to reveal such a specific antineuronal antibody in sera of many myasthenics representing various ages and duration and severity of disease, making us skeptical of that other report.

We are seeking the putative factor(s) evolved from the thymus which is detrimental to neuromuscular function in MG patients, by incubating in a tissue culture medium thymus removed at time of therapeutic thymectomy of our patients. (In the previous year we also received some thymuses through the kindness of Gabriel Genkins and Joseph Peabody.) Last year we stated that our initial experiments seemed to show an inconstant blocking effect in the rat neuromuscular preparation. This year, though, we have found a source of experimental error to explain that effect, and since correcting the error we have not yet found blocking action. We are, therefore, modifying our incubation technique and increasing the sensitivity of the assay model.

Significance to Bio-Medical Research and the Program of the Institute: The response of myasthenic patients to alternate-day prednisone indicates that much of the abnormality is not irreversible, and it supports the idea that improvement can result even in the most severely affected and advanced stages of this disease. The demonstrated benefit of alternate-day prednisone in juvenile myasthenia gravis led us to apply this new program to adult myasthenia gravis patients. It has resulted in a remarkable new method of treatment which is now widely used throughout the world. Advantages of this prednisone program in the treatment of myasthenia gravis include its being a non-surgical means of providing lasting improvement by an oral medication not requiring administration at rigid intervals during the day, not producing an

initial drug-induced worsening, while allowing continuous long-term use, and with its effect thought to be anti-pathogenic rather than anti-symptomatic. We now consider alternate-day prednisone the most effective treatment in myasthenia gravis and believe it offers the possibility of suppressing the disease effectively for a sufficiently long period for it to disappear completely. If this will occur, the disease would be cured in such a patient.

Lactate infusion seems to be a new and relatively safe test for helping to diagnose myasthenia gravis. The other findings described herein help shed light on the pathogenesis of myasthenia gravis.

Proposed Course of Project: The various clinical and basic mechanistic aspects of our new alternate-day prednisone program will be investigated more extensively, and predictive parameters sought to help anticipate which patients will respond and how much drug they will need. The detrimental substance(s) from the MG thymus will be further sought. Other facets of the pathogenesis and etiology will be explored.

Honors and Awards: None

Publications:

Patten, B. M., Oliver, K. L. and Engel, W. K.: Adverse interaction between steroid hormones and anticholinesterase drugs. Trans. Amer. Neurol. Assn. 98:248-252, 1973.

Patten, B. M., Oliver, K. L. and Engel, W. K.: Effect of lactate infusions on patients with myasthenia gravis. Neurology, 1974, in press.

Patten, B. M., Oliver, K. L. and Engel, W. K.: Adverse interaction between steroid hormones and anticholinesterase drugs. Neurology 24:442-449, 1974.

Engel, W. K., Festoff, B. W., Patten, B. M., Swerdlow, M. L., Newball, H. H., and Thompson, M. D.: Myasthenia gravis. Ann. Intern. Med., 1974, in press.

Serial No. NDS(I)-65 MN/OC 1191(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunological Abnormalities of Neurologic Diseases

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
John N. Whitaker, M.D.
John L. Trotter, M.D.
Jay D. Cook, M.D.

Other Investigators: Warren Strober, M.D.
Jerry R. Mendell, M.D.
P. V. Holland, M.D.
H. J. Alter, M.D.

Cooperating Units: Department of Neurology, V.A. Hospital, Memphis, Tenn.
Metabolism Branch, NCI
Blood Bank, Clinical Center
Ohio State University School of Medicine, Columbus, Ohio

Man Years:

Total:	2.6
Professional:	2.2
Other:	0.4

Project Description:

Objectives: To apply a variety of basic immunological techniques to the study of human diseases affecting the neuromuscular system and central nervous system.

Methods Employed: Various basic immunochemical and immunocellular techniques were employed for studying both immunoglobulin and cellular aspects of altered immune states.

Patient Material: Specimens of peripheral blood, urine, cerebrospinal fluid (CSF), bone marrow, muscle, peripheral nerve, thymus and other tissue was obtained as part of diagnostic or therapeutic procedures from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: Our immunology laboratory has been re-established on a very modest basis, with capabilities in a variety of sophisticated basic techniques, using highly purified immunoreagents we have made ourselves (with NCI).

An interesting finding we reported was that 6 of 7 patients of a family with hereditary sensory neuropathy had elevated immunoglobulin A in their serum. Studies to determine the cause of the increased levels of serum immunoglobulin A were done in collaboration with the Metabolism Branch, NCI. Three of four patients affected with the hereditary sensory neuropathy had elevated small-bowel-production of immunoglobulin A; one affected patient and two unaffected siblings had normal small bowel immunoglobulin A production levels. Although this finding does not at the present time explain the cause of the neuropathy, it may add significant information to understanding possible pathogenetic mechanisms and has prompted a new hypothesis concerning the mechanism of their neuropathy: possible familial susceptibility to infection in the gut tissue (in which there are excessive eosinophils, suggesting a local immunologic reaction) that causes through, or parallel to, the IgA overproduction a metabolic abnormality damaging sensory nerves (see also our Amyotrophic Lateral Sclerosis Project).

Serum immunoglobulin and $B_{1c/1A}$ globulin levels have been determined on a large group of patients from the Medical Neurology Branch. A number of patients with a chronic peripheral neuropathy currently considered to be idiopathic were reported to have an elevated immunoglobulin M, suggesting a previously unsuspected immunologic pathogenesis in them.

As part of the same study in which immunoglobulin and complement levels were studied in patients, immunoelectrophoretic determinations were done on all the patients admitted to the Medical Neurology Branch. In one patient with adult-onset rod neuromuscular disease, an unusual paraproteinemia was found, consisting of a IgG/lambda myeloma protein. He appears to have had a monoclonal gammopathy for over a year, without detectable evidence of myeloma. The possible relationship between the paraprotein and the neuromuscular disease is being explored. In his biopsy, we recently found rods in the muscle nuclei that are ultrastructurally identical to those within myofibrils (see our Myopathy Project).

The binding site of the anti-muscle factor highly characteristic of myasthenia gravis (MG) (which we previously showed was an IgG and true antibody), we have reported to be localized with immunohistochemistry-electronmicroscopy to the sarcoplasmic reticulum, but only the reticulum at the I-band level. This binding, incidentally, demonstrates for the first time a chemical-structural specialization of the reticulum of the I-band in contrast to that of the A-band. The binding and its localization does not immediately explain the cause of the severe weakness of MG, but it provides another important and nearly disease-specific lead to be followed-up (see our Electronmicroscopy Project).

The site of the abnormality in myasthenia gravis causing the neuromuscular weakness is still undetermined. Our own evidence and that of others makes us

feel that the neuron and presynaptic mechanisms are likely to underlie the myasthenia gravis defect. Because of this, the studies previously done by another worker, in which he found antineuronal binding of sera from patients with myasthenia gravis, seemed very important but needed to be confirmed and possibly extended. He suggested that the factor was neuron-specific, in its binding to nuclei of neurons only, except for spermatogonia binding too. In our indirect immunofluorescent study of 15 patients with myasthenia gravis, binding of antibodies to neurons (to their nuclei) of the IgG, IgA and IgM classes was found only in those patients who also had antinuclear antibody in these classes. Additionally, patients with systemic lupus erythematosus, who did not have myasthenia gravis but who did have antinuclear factor, had the same spectrum of staining patterns. Therefore, we concluded and reported that the neuronal nuclear binding really represents part of the antinuclear factor activity sometimes present in the sera of patients with myasthenia gravis and is neither neuronal-specific nor a phenomenon specific to that disease.

Our finding of immunoglobulin complexes deposited in blood vessels in 83% of the childhood cases and 29% of adult cases, reported previously, has now been confirmed by others. This supported our earlier hypothesis that childhood polymyositis/dermatomyositis (PM/DM) may be another form of blood vessel abnormality in muscle, on the venous side and perhaps to some extent also on the arterial side. Since this and other immunologic abnormalities of PM/DM could in turn be caused by a viral infection, search for Australian antigen (hepatitis B antigen), circulating or bound to blood vessels or muscle cells, was made, but we have reported it to have been completely negative (with CC).

Attempts have been made by various manipulations to grow a virus from dermatomyositis/polymyositis muscle, with negative results to date (with NIDR).

We have reported our findings and compared them with ones of other investigators to propose that the childhood form of dermatomyositis/polymyositis may have a different emphasis of pathogenesis from adult forms of the disease complex, the former being related more to immunoglobulin complexes and the latter more to lymphocytotoxicity.

Specimens collected from every Medical Neurology Branch patient and stored in our serum and spinal fluid bank have proven to be valuable in the study of a number of entities of possible viral and/or autoimmune etiology.

Significance to Bio-Medical Research and the Program of the Institute: Since myasthenia gravis, dermatomyositis/polymyositis, and some types of progressive or relapsing polyneuropathies are probably on a dysimmune basis, analysis of the details of the abnormal immune process is expected to help formulation of better methods of treatment and prevention.

Proposed Course of Project: Ideally, all the immunologic studies noted herein should be elucidated more fully with the best available immunological techniques. However, with our extremely limited resources we are restricted in what we can do.

Honors and Awards: None

Publications:

Whitaker, J. N., Holland, P. V., Alter, H. J. and Engel, W. K.: Idiopathic inflammatory myopathy. Arch. Neurol. 28:410-411, 1973.

Whitaker, J. N., Sciabbarrasi, J., Engel, W. K., Warmolts, J. R. and Strober, W.: Serum immunoglobulin and complement (C3) levels in adults with idiopathic chronic polyneuropathies and motor neuron diseases. Neurology 23:1164-1173, 1973.

Whitaker, J. N., Falchuck, A. M., Blaese, R. M., Engel, W. K. and Strober, W.: Disorders of the immunoglobulin A system in hereditary sensory neuropathy. Trans. Amer. Neurol. Assn. 98:60-63, 1973.

Mendell, J. R., Whitaker, J. N. and Engel, W. K.: The skeletal muscle binding site of anti-striated muscle antibody: An ultrastructural study using peroxidase conjugated antibody. J. Immunol. 111:847-856, 1973.

Whitaker, J. N. and Engel, W. K.: A search for antibodies to neuronal nuclei in the serum of patients with myasthenia gravis. Neurology 24:61-63, 1974.

Whitaker, J. N. and Engel, W. K.: Mechanisms of muscle injury in idiopathic inflammatory myopathy. New Engl. J. Med. 289:107-108, 1973.

Whitaker, J. N., Falchuck, Z. M., Engel, W. K., Blaese, R. M. and Strober, W.: Hereditary sensory neuropathy. Arch. Neurol. 30:359-371, 1974.

Serial No. NDS(I)-65 MN/OC 1192(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electronmicroscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Adam N. Bender, M.D.
Valerie Askanas, M.D., Ph.D.

Other Investigators: Jerry R. Mendell, M.D.
John N. Whitaker, M.D.
Husseiny I. Dessouky, M.D.
John W. Griffin, M.D.

Cooperating Units: Muscle Research Laboratory, Institute for Physical Medicine and Rehabilitation, N.Y. University School of Medicine, N.Y., N.Y.
Division of Neurology, College of Medicine, The Ohio State University, Columbus, Ohio
Department of Neurology, V.A. Hospital, Memphis, Tennessee
N.I.H.L.

Man Years:

Total:	3.1
Professional:	2.1
Other:	1.0

Project Description:

Objectives: To study the early subcellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle, such as denervating and metabolic diseases. To study subcellular changes of human neurons and peripheral nerves in certain diseases.

Methods Employed: A portion of patient diagnostic biopsy muscle is placed in our specially-designed clamp to maintain resting length and is immediately fixed in cold buffered glutaraldehyde or osmium tetroxide for eventual araldite or epon embedding. Other fixative combinations and buffers

(including cacodylate), and various electronmicroscopic (EM) staining procedures are used. EM histochemistry for acid phosphatase, cytochrome oxidase, butyric dehydrogenase, calcium and an ATPase have been done on human muscle. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in the Histochemistry Project. Histochemically stained cryostat sections, ready within half an hour after the biopsy, are used to determine which of the biopsies contain light microscopic features of special interest and thereby guide choosing material appropriat for electronmicroscopic study and for directing the search for ultra-structural abnormalities. If necessary, a repeat biopsy of a patient can be done by needle technique to obtain additional tissue for EM. Biopsy specimens of human nerve or brain tissue are fixed and embedded for electronmicroscopy and are similarly monitored by rapid enzyme histochemistry of adjacent tissue. Normal and abnormal human muscle grown in tissue is also studied by EM (for new techniques, v.i.). Skin samples and cultured fibroblasts from our patients are sometimes studied, as are mitochondrial preps of sarcolemmal membrane from our biochemical studies. In the animal models and tissue cultures studied in parallel to the human diseases, marker substances such as peroxidase, ferritin, ruthenium red, and lanthanum are sometimes used with electronmicroscopy.

Patient Material: Patients with various myopathies and neurogenic forms of muscular weakness are biopsied for diagnostic purposes. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Muscle biopsies and nerve biopsies are the major tissues examined. Muscle from patients is sometimes grown in tissue culture for a few weeks before being studied by EM. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease. Other abnormal tissues from neurologic patients are sometimes examined, such as skin and cultured fibroblasts.

Major Findings: Electronmicroscopy of muscle fibers cultured from our patients has been focussed on the problem of seeking whether biopsies from patients with "ragged-red" and "rugged-red" fibers (ones with abnormal, bizarre mitochondria) reproduce that change when growing in vitro, free of other body influences. The biopsies are prepared in our tissue culture laboratory and sent to Dr. Valerie Askanas in New York for culturing and histochemical or EM staining, and then embedding, cell-selection and blocking, with the subsequent sectioning and electronmicroscopy then being done in our EM laboratory. This system has now been well worked out logistically, and it will be even more precisely coordinated when the sectioning can be done there and the sections brought here by Dr. Askanas for her to study by EM with us. And because abnormalities in human disease often affect only an occasional fiber or part of a fiber we have developed with Dr. Askanas, a new technique for embedding whole cultures, prestaining for EM-histochemistry and for cell identification, and quickly and precisely getting out (on the basis of selection by light-microscopy) those individual muscle fibers of greatest potential EM interest. To date we have demonstrated good growth of ragged-red and rugged-red fiber biopsies in culture, and have obtained fibers with

mitochondria nicely stained by the EM cytochrome oxidase method, and have found suggestive abnormalities of mitochondria. (Details of all these technical advances have been prepared for publication -- see also our Tissue Culture Project.) We are also attempting, with Dr. Askanas, to produce ragged/rugged-red fiber kind of mitochondrial abnormalities by applying to tissue cultured normal human and animal muscle various drugs, and then looking at them with EM.

EM studies of cultured muscle from other patients with various neuromuscular diseases are in progress, such as ones with bizarre polysaccharide storage in muscle.

In myasthenia gravis, the precise binding site of the highly disease-characteristic serum muscle-binding factor (Strauss, et al.), which we have previously shown to be an immunoglobulin G and true antibody, was localized by electronmicroscopy-immunology. Using the direct Coons technique and peroxidase-labeled reagent, it was reported that the serum anti-muscle factor binds only to the sarcoplasmic reticulum of the I-band in the muscle fiber, and not to the reticulum of the A-band. This is the first demonstration of a structural-chemical difference between the reticulum of the I- vs. A-band.

"Ragged-red fibers" is now a generally used term we introduced to describe the histochemical appearance of abnormal muscle fibers which contain collections of large, bizarrely-structured mitochondria by EM. We have now observed such fibers in a new hereditary syndrome consisting of limb-girdle muscular "dystrophy", myoclonus epilepsy and elevated basal lactates and pyruvates that is often fatal in childhood (see our Myopathy Project). We have also introduced the name "rugged-red fibers" for ones containing many of the same mitochondrial abnormalities but less overall fiber abnormality. In one patient with rugged-red fibers, who also had mild muscle weakness and cardiac idiopathic septal hypertrophy (with NHLI), numerous light-cored dense particles were found in numerous structurally abnormal mitochondria. These particles resemble ones induced experimentally by calcium abnormalities and also have some resemblance to viral particles (in which case they might be considered a sort of "mitochondriophage").

Histochemical techniques for cytochrome oxidase, beta-hydroxy-butyric dehydrogenase, and acid phosphatase at the EM level have now been established in the laboratory and are being used on the human muscle biopsies. The initial objective has been to delineate the bizarre mitochondrial changes present in "ragged-red fibers." We have shown that the intramitochondrial crystals are typically not stained by the ultrastructural cytochrome oxidase reaction.

In two patients with late-onset rod disease (an electronmicroscopically delineated entity we have originally described previously), one of whom incidentally was found to have a monoclonal gammopathy (IgG/lambda myeloma protein in the serum), we have delineated by EM intranuclear rods morphologically identical to those of the cytoplasm. This discovery provokes the

alternative hypotheses of nuclear rod pathogenesis as formed from either (a) hitherto "neglected" nuclear contractile protein (? actin-like, decorated with alpha-actinin) or (b) those cytoplasmic contractile proteins "imported" into the failing nuclei, a general pathologic mechanism not hitherto discussed in any disease.

A new structural abnormality of human muscle fibers has been identified by light-microscopic histochemistry and by EM. It is tentatively called "zipper tubules" and exists only in the type I fibers.

Electron micrographs have been made on a number of our other patients and are being correlated with all our other studies.

We have begun to seek and find end-plates in our EM studies of human muscle. First pictures suggest some may be abnormal in central core disease -- such a finding is in harmony with our hypothesis, based on our other EM and histochemical studies, that central core disease is a disorder of the lower motor neurons.

By electronmicroscopy, combined with histochemistry, 3 more cases of what we originally described as focal loss of cross-striations have been identified. The original case has been described in full EM and histochemical detail in a chapter in "Shy's Neurology" (an in-press book).

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases is being conducted, in an attempt to confirm one report by another group. To date, "myxovirus-like" structures have been found in some cases of polymyositis, but their significance remains unknown, since other investigators are finding them in various other diseases.

The degree of "purity" of cell fractions (membrane preps) used in our biochemical studies (see our Biochemistry Project) have been monitored by electronmicroscopy.

EM autoradiographic study of transfer of "putative trophic molecules" from lower motor neuron axon to schwann cell or to muscle fiber is discussed in our Autoradiography Project.

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its childhood. Identification of new forms of disease still occur rapidly in this stage. However, one must be careful not to name a condition on the basis of limited, though careful, EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is close correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, as in semi-crystalline bodies, perhaps the light-cored dense particles, and other changes of mitochondria. Certain other ultrastructural changes, e.g., accumulation of lipid lysosomes, or glycogen in fibers provide clues as to which metabolic pathways are likely to be the site of a presumed enzyme defect.

These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. Perpetuation of, or production of, characteristic ultrastructural abnormalities of human muscle cells in tissue culture should help clarify disease mechanisms. Electronmicroscopy is also indispensable for the detailed morphologic analysis of topics listed under our other projects, e.g., the viral-etiology hypothesis of polymyositis and dermatomyositis, monitoring purity of cell fractions studied biochemically, and localization substances by EM-autoradiography.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic, viral, and tissue culture techniques with electronmicroscopy applied to human neurologic diseases and animal models thereof have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Honors and Awards: None

Publications:

Mendell, J. R., Whitaker, J. N. and Engel, W. K.: The skeletal muscle binding site of anti-striated muscle antibody: An ultrastructural study using peroxidase conjugated antibody. J. Immunol. 111:847-856, 1973.

Engel, W. K.: Central core disease and focal loss of cross-striations. In Shy, G. M., Goldensohn, E. S. and Appel, S. H. (eds.): The Cellular and Molecular Basis of Neurologic Disease, Philadelphia, Lea and Febiger, 1974, in press.

Serial No. NDS(I)-65 MN/OC 1193(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
John W. Griffin, M.D.

Other Investigators: Barry W. Festoff, M.D.
Malcolm C. Johnston, M.D.
Donald Price, M.D.

Cooperating Units: Human Genetics Branch, NIDR
Department of Neuropathology, Johns Hopkins School of Medicine, Baltimore, Md.

Man Years:

Total:	0.7
Professional:	0.6
Other:	0.1

Project Description:

Objectives: To apply radioautographic techniques to study structure and function in normal and abnormal neurons and muscle cells of man and of animal models of human disease.

Methods Employed: Standard radioautographic methods, primarily using emulsion-dipped slides. Various histochemical enzymatic counterstains for end-plate and muscle fiber components are done after photographic development of the slides, such as esterase to show location of motor end-plates. A number of samples are also prepared for electron-microscopic autoradiography. Application of isotope is by one of several methods: injection of tritiated leucine into rat anterior horns to study axoplasmic flow and transfer; labelled α-bungarotoxin or labelled anti-α-bungarotoxin antibodies applied to sections of animal or human muscle biopsy tissue; "pulse-feeding" cultured human muscle or animal muscle or neurons various labelled substances.

Patient Material: Muscle biopsies obtained from our patients for diagnostic purposes -- additional sections are cut from the fresh-frozen blocks for incubation with various radioisotopes, e.g., ^{125}I-α-bungarotoxin. Some human muscle is tissue cultured before autoradiographic study.

Major Findings: A method has been obtained for demonstrating localization of ^{125}I-α-bungarotoxin (which we have highly purified) to the cholinergic receptor molecules neuromuscular junction, which is made manifest by histochemical post-staining for acetylcholinesterase. This technique is now being applied to various human diseases known or suspected to be related to abnormality (a) at the neuromuscular junction, or (b) of the nerve influence on muscle. The latter is expected to allow spread of α-bungarotoxin receptors away from the normal narrow confines of the neuromuscular junction. The progress has been slow this year because of lack of our own autoradiography facility and a technician -- we were borrowing those amenities from NIDR for the α-bungarotoxin work.

Also borrowed (from Johns Hopkins School of Medicine) are the facilities for EM-autoradiographic studies of axon-to-schwann-cell and axon-to-muscle transfer of ^{3}H-leucine-labelled proteins. Rat lower motor neurons (LMNs) are labelled by stereotactic injection of ^{3}H-leucine into the anterior horns. The distribution of tritiated proteins at 6 and 48 hours and 5 and 7 days is evaluated by autoradiographic and scintillation counting techniques. Intra-axonal label was present in the sciatic nerves at all times studied. Preliminary results demonstrate distinct label in axonal presynaptic endings at the neuromuscular junctions. The time course of appearance and disappearance will be followed, and factors influential thereon will be studied. Transfer of those axonal molecules to muscle will continue to be sought with more refined methods. Scintillation counting suggests label in muscle, but it has not yet been localized by autoradiography. We have also demonstrated appearance of labelled protein in schwann cells of sciatic nerve after ^{3}H-leucine injection into the anterior horns. Studies designed to distinguish between axon-to-schwann-cell macromolecular transfer vs. re-utilization of labelled leucine are in progress -- if the former is true it will support our hypothesis of several years ago regarding a trophic influence of axon on schwann cells.

We are also developing techniques to study autoradiographically in vitro metabolic parameters of nerve fascicles taken during diagnostic nerve biopsy from patients with various abnormalities affecting their peripheral nerves.

Finally, we have developed plans to combine autoradiographic techniques with our cultures of human skeletal muscle from patients with various types of disease and also with our cultures of animal neurons and muscle. We are delayed in this by lack of any space and technical help of our own in autoradiography.

Significance to Bio-Medical Research and the Program of the Institute: (a) Several human neuromuscular diseases are still controversial regarding their neurogenic vs. myogenic pathogenesis. The evaluation of ACh receptor molecules might help identify various diseases with subtle impairment of neural control of muscle. (b) Details on the type of trophic control of axon on schwann cells and muscle fibers, and the molecules involved, if we can

clarify them, may have broad implications for understanding various neuromuscular diseases and "peripheral neuropathies." (c) Autoradiographically obtained information on human normal and diseased muscle fiber metabolism and animal neuronal and muscle fiber metabolism will have important bearing on a number of human neuromuscular diseases.

Proposed Course of Project: Extend our preliminary studies to various human neuromuscular diseases in a more complete and systematic manner, and extend our preliminary animal model studies, as discussed above. We definitely need to obtain an additional room and a technician to do all the work in our own Branch.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 MN/OC 1728(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Brain Tumor

Previous Serial Number: Same

Principal Investigators: Darell D. Bigner, M.D.
John P. Kvedar, A.B.
W. King Engel, M.D.
Nicholas A. Vick, M.D.

Other Investigators: W. Ray Bryan, Ph.D.

Cooperating Units: Viral Oncology Branch, NCI
Duke University School of Medicine,
Department of Neurology, Durham, N.C.

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: (1) To develop a brain tumor animal model in which to study the pathogenesis and better methods of diagnosis of brain tumors; (2) to develop the most sensitive animal system in which to attempt recovery of oncogenic viruses from human brain tumors; (3) to improve methods of virus extraction from SR-Rous sarcoma virus (SR-RSV) tumors; and (4) to develop new methods of treating brain tumors.

Methods Employed: (a) Assay of SR-RSV, an RNA tumor-inducing virus, on the chorioallantoic membrane of chicken eggs; (b) preparation of high-potency, bacterial-free suspensions of SR-RSV by a new combination of buffer systems, differential centrifugation, and membrane filtration; (c) induction of brain tumors in young dogs or hamsters by injecting the high-potency suspensions of SR-RSV; (d) measurement of antibody responses in tumor-bearing dogs, demonstration of SR-RSV antigen in the induced brain tumors; (e) recovery of SR-RSV from the tumors in chick embryo fibroblast tissue culture, in chicken, or in Japanese quail; (f) measurement of the effect of various drugs on reducing the amount of SR-RSV necessary from brain tumor induction; (g) morphologic study with histochemical, electron microscopic, light microscopic, and

immunofluorescent techniques, seeking (1) viral particles and (2) details of the morphologic changes of the neoplastic cells; (h) brain scanning of tumor-bearing animals with various isotopes.

Patient Material: None

Major Findings: Published last year (Bigner, D.D., Kvedar, J.P., Shaffer, T.C., Vick, N.A., Engel, W.K. and Day, E.D.: Factors influencing the cell type of brain tumors induced in dogs by Schmidt-Ruppin Rous sarcoma virus. J. Neuropathol. Exp. Neurol. 31:583-595, 1972) was a detailed account of this work, covering the following data. An improved method of concentrating and purifying Schmidt-Ruppin Rous avian sarcoma virus (SR-RSV) yielded, 4 weeks after intracerebral inoculation into newborn dogs and hamsters, 96% survivor incidence and 100% brain tumor incidence in survivors, compared to 49% and 86% respectively with previous methods. A quantitative dose-response relationship was demonstrated. The tumors were sarcomas and gliomas (spongioblastomas, astrocytomas, and ependymomas) with varying degrees of anaplasia. With a smaller (0.01 ml) inoculum volume of the most highly concentrated SR-RSV, brain inoculation site was the determinant of type of tumor induced; 7/10 dogs inoculated in the subependymal cell plate developed only gliomas, and all dogs in which tumors were induced after inoculation in the parietal subdral space (5/5) or the cerebellar vermis (4/5) developed sarcomas. No evidence of virus was found in dog brain tumors with electron microscopy or immunologic methods, but the SR-RSV genome was detected by inoculating whole cell suspension of dog and hamster brain sarcomas and gliomas back into avian hosts, wherein typical Rous sarcomas containing C-type viral particles, group specific viral antigens, and infectious RSV were induced.

Significance to Bio-Medical Research and the Program of the Institute: These induced brain tumors are an especially suitable system in which to study mechanisms that allow malignant transformation of glial and mesodermal brain cells by RNA-C-type viruses without detectable evidence of viral replication, as is likely to be occurring in at least some spontaneous human brain tumors. This system will serve as a guide to isolation of viruses causing human brain tumors. The reproducibility and rapidity of experimental tumor induction, type of blood supply, and histologic similarity of the experimental gliomas to spontaneous human gliomas make these SR-RSV-induced animal brain tumors ideal for morphologic, chemotherapeutic and brain-scanning studies. They are now being used to study (with LNNS, IR) the blood-brain-barrier changes in tumors, combining tracer substances with electron microscopy.

Perhaps the most important implication is the fact that virally induced brain tumors that have no evidence of viral particles or viral antigens, but which do contain viral genetic material, are available for study, since these same conditions may be operative in spontaneous human brain tumors.

Proposed Course of Project: Ideally the project should be continued in several directions. First, the technology of concentrating SR-RSV should be extended to extraction of spontaneous human brain tumors for inoculation of animals to try to demonstrate the viral etiology of human tumors. In addition, the basic cellular mechanisms that allow malignant transformation of cells with our detectable evidence of viral antigens or mature virus should be studied and applied to the human situation. Also, the ability to induce large brain tumors in large and small animals should be exploited for evaluating chemotherapeutic agents and better diagnostic methods, such as brain scanning.

However, since neither positions nor space has been granted for this project, it has been discontinued in this Branch.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 MN/OC 1792(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrophysiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Richard B. Rosenbaum, M.D.

Other Investigators: John R. Warmolts, M.D.
M. Zafar Mahmud, M.D.
Bernard M. Patten, M.D.

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	1.7
Other:	0.0

Project Description:

Objectives: (1) To develop more precise methods of electromyography (EMG) recording and analysis; (2) to establish better EMG indices of early myopathic or neuropathic involvement; (3) to improve the value of the EMG in distinguishing the neural vs. myopathic basis of atypical disorders; (4) to investigate mechanisms regulating motor unit recruitment in normal subjects and those with neuromuscular disorders; (5) to correlate the electrical behavior of motor units with their histochemically defined properties; (6) to use clinical electrophysiologic parameters to evaluate course of disease and response to treatment; (7) to use electrophysiologic techniques to analyse induced animal-models of human neuromuscular diseases.

Methods Employed: Recordings are made in humans using the Teca-Medelec electrophysiologic analysis system. Permanent data are stored by high-speed magnetic tape recording and photographic recording. Correlation studies involve the (a) recruitment threshold, (b) rank order of recruitment, (c) preferential stable discharge frequency, (d) relative rhythmicity, (e) amplitude, (f) duration, and (g) configuration of EMG recorded individual motor unit action potentials in human subjects with a variety of neurogenic and myopathic conditions, and with neuromuscular disease of uncertain type, as well as in subjects without definable neuromuscular disease. We continue

to use a method we previously developed for recording electrophysiologically in the operating room during open biopsy and excising the region of fibers directly around the tip of the EMG electrode ("Open Biopsy EMG").

A variety of parameters of neuromuscular transmission are repeatedly evaluated in individual myasthenia gravis patients before and during treatment. Partial curarization of subjects with myasthenia gravis and other fatigue states are performed with special attention directed to the behavior of single motor units.

Electrophysiologic abnormalities are sought in various systemic diseases affecting the neuromuscular system, such as hyperparathyroidism and non-metastatic distant cancer, and correlated with clinical and histochemical data (see out Amyotrophic Lateral Sclerosis Project).

Patient Material: Patients with a variety of neuromuscular disorders and without definable neuromuscular disease from the Medical Neurology Branch and the neurologic consultation service we provide throughout the Clinical Center, in whom EMG studies are being done for diagnostic purposes.

Major Findings: Several new aspects of clinical electromyography (EMG) have been published this past year. Our previously introduced new descriptive term for the pattern of short duration, small amplitude, excessively abundant potentials for a given amount of voluntary effort, viz., "SSAP", used to replace the generally employed term "myopathic" (a designation which we consider to be misleading at times) was modified in acronym (but not in substance). The "short" was changed to "brief", i.e., "BSAP", allowing one to use the two subacronyms of BAP and SAP when the situation applies. We consider BSAPs just as likely to be due to neuronal disease as to myopathic disease (v.i.).

In using the technique we introduced and previously published, "Open Biopsy Electromyography" (whereby one is able to obtain tight correlation of electrophysiology done by EMG recording of the same muscle fibers excised immediately after recording and studied histochemically) to study patients whose biopsies showed predominance of either type I or type II muscle fibers, we have identified the different physiological properties of corresponding type I and type II human motor units. Human type I units in the limb muscles studied fire first during minimal voluntary contraction and they fire at a lower frequency, 6-10 hertz in a sustained rhythmic fashion (but could increase to five in a steady fashion as high as 18-20 hertz on full voluntary effort). Type II units fire only with a hard quick contraction, fire faster than from 10-25 hertz, in a brief unsustained unrhythmic fashion (with peak firing frequencies ranging from 16-50 hertz during the height of the bursts during the most vigorous contractions). Thus standard electromyography techniques for recording motor unit potential duration and amplitude done with mild voluntary contraction for clarity of the trace probably record only the behavior of type I units.

The electromyographic patterns of selective type II and type I muscle fiber atrophy in humans have been identified, and an hypothesis developed that relates the electrical data to the possible neurogenic origin we propose may be operative in these atrophies.

In lectures and writings we have continued our "campaign" against designation of the BSAP EMG pattern as "myopathic" -- nevertheless, many authors in a steady flow of articles still continue not only to write "myopathic" but, worse, to think that the BSAP pattern is diagnostic exclusively of a myopathy. We continue to propose, on what we consider very sound theoretical grounds, that the BSAP EMG merely denotes either (a) small (few-fiber or "hypocomplemented") motor units of type I (or both types of) or (b) motor units eucomplemented but with smaller-than-normal-diameter fibers. The hypocomplemented units could be due either to a defect in development ("hypoplastic units") or acquired fractionation. Although any of these changes theoretically could be myopathic, any could also be neuropathic. We therefore conclude that the BSAP EMG pattern is absolutely non-diagnostic and that there is nonesuch animal as a "myopathic EMG".

In our formulation that abnormality of the lower motor neuron or its axonal branches cause a BSAP pattern, we hypothesize, on the basis of correlated electromyographic and histochemical study and in comparison to theoretical possibilities, that a neural abnormality is the basis of all or most of the muscle abnormality in the following diseases with BSAP EMGs: myasthenia gravis, central core disease, myotonic atrophy (formerly called myotonic dystrophy), myotonia congenita, paramyotonia congenita, type-I-fiber-hypotrophy-with-central-nuclei, type-I-fiber-smallness-without-central nuclei, and the form of benign congenital hypotonia with type I fiber predominance (see our ALS, Myopathies and Myasthenia Gravis Projects).

Using a micromarking technique we adapted for open biopsy electromyography, we have verified in human limb muscle that motor end-plates are the signal source for what has been postulated to be "end-plate noise", through the use of cholinesterase staining of structures marked after their emanating "end-plate noise" was recorded. With this technique, studies of microscopic morphology as correlated with cholinesterase-staining, histochemical fiber type, and nerve terminal proliferation as demonstrated by axon silver staining, can be correlated in normal humans and ones having various neuromuscular diseases with behavior of miniature end-plate potential activity in response to pharmacologic agents.

In a number of systemic diseases, the presence and type of neuromuscular involvement was identified by EMG correlated with histochemistry. For example, in hyperparathyroidism (with NIAMD), we reported that nearly all of 16 patients were considered to have mild to moderately-severe lower motor neuron involvement, sometimes mimicing aspects of amyotrophic lateral sclerosis (see our ALS Project). In very early, non-metastatic, distant cancer most patients had slight or moderate evidence of type II fiber atrophy that was, at least in most cases, probably based on motor neuron involvement (with NCI) (see our ALS Project). In xeroderma pigmentosum peripheral neuropathy was identified (with NCI) (see our ALS Project).

Normal standards for voluntary action potentials and nerve conduction studies for this laboratory are being collected.

Diagnostic electrophysiologic studies are provided as part of our consultation service to other clinical units of the Clinical Center.

Significance to Bio-Medical Research and the Program of the Institute: Treatment of the various, and numerous, human neuromuscular diseases is based on precise diagnosis and understanding of the pathophysiology. As a group, human diseases of the lower motor neurons (including peripheral neuropathies) and muscle are just beginning to have their pathophysiology partly understood. The detailed neurophysiologic techniques herein described are leading to more precise delineation of these diseases, and in some instances causing us to completely change our concepts of their pathogenesis. Investigation of the pathogenesis is also partly dependent on knowing whether a disease is primarily one of motor neuron or muscle -- for example, it would be useless to do biochemical assays on muscle homogenates of central core disease if the disorder is essentially one of the lower motor neuron.

EMG is also important for detecting subclinical presence or dissemination of neuromuscular disease, and its type, in patients with various generalized disorders, e.g., distant cancer, hyperparathyroidism, or xeroderma pigmentosum.

We believe that if we can eliminate the term "myopathic EMG" or "the EMG was myopathic" from neurologic jargon, and, more importantly, from the neurologic "thinking", clinicians and investigators will have a freer approach to ponder the possible pathogenesis of various ill-understood neuromuscular diseases.

We are also now learning about different physiological properties of the various types of human motor units, which may lead to a better understanding of their involvement in various neuromuscular diseases.

Proposed Course of Project: This project will be continued and elaborated upon, e.g., with even more detailed correlation between EMG and histochemistry in patients with various diseases.

Honors and Awards: None

Publications:

Warmolts, J. R. and Engel, W. K.: Correlation of motor unit behavior with histochemical myofiber type in humans by open-biopsy electromyography. In Desmedt, J. E. (ed.): New Developments in EMG and Clinical Neurophysiology, Basel, S. Karger, 1973, pp. 35-40.

Engel, W. K.: "Myopathic EMG" -- nonesuch animal. New Engl. J. Med 289:485-486, 1973.

Serial No. NDS(I)-62 MN/AP 926(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: The sequential pattern of physiological events which develop in skeletal muscle starting with excitation and ending in relaxation has not been adequately defined. The molecular interactions that link excitation to contraction in the physiological state are largely unknown. Not much knowledge has accumulated about the molecular changes involved in the uncoupling of contraction from excitation which permits relaxation. The objective was to establish an ionic basis for initiation, maintenance, and subsequent loss of tension in slow-type skeletal and in smooth muscle. An additional objective was to relate the ionic events to electrical, mechanical and metabolic activity and to determine how these parameters were related to drug action.

Methods Employed: The coupling and uncoupling processes between the tension-relaxation cycle and the related ionic events are investigated in smooth muscle and in slow-type skeletal muscle. Slow skeletal muscle has now been found to occur in many mammalian species and smooth muscle occurs in all mammals. Since classical electrophysiological and mechanical methods have failed to explain the coupling process ion substitution methods have been developed and used. Sucrose gap techniques have been modified and used for monitoring membrane potential changes. Ion contents are measured by atomic absorption spectrophotometry. Dimonovalent di-buffer amines have been developed and used as monovalent, nonpenetrant, and electrical charge substitutes for Na^+ and Cl^-.

Major Findings: Last year it was stated that examination of ion content data gathered under conditions of Ca^{++} lack has verified that increased Na^{+} entry in slow skeletal relates to teh development of contracture tension. This verification is in accordance with the concept that in this type of muscle a Na^{+} transport system functions to maintain the integrity of muscle function. Similar conditions are probably existent in smooth muscle but more rapid ion transport kinetics have precluded definitions. This year the project has been inactive because the Principal Investigator is on detail as the Assistant Director of the Intramural programs.

Significance to Bio-Medical Research and the Program of the Institute: This project furnishes new data of a basic nature that relates to muscle function and malfunction. Knowledge about smooth muscle may help explain how vascular smooth muscle is involved in stroke mechanisms or the etiology of Duchenne dystrophy.

Proposed Course of Project: This year the project has been inactive because the Principal Investigator is on detail as the Assistant Director of the Intramural programs.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1049(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

Previous Serial Number: Same

Principal Investigators: Jay B. Wells, Ph.D.
Mark Schoenberg, M.D.

Other Investigators: None

Cooperating Units: Section on Cellular Physics, Laboratory of Physical Biology, NIAMD

Man Years:

Total:	1.8
Professional:	1.8
Other:	0.0

Project Description:

Objectives: The general purpose of the project continued with the investigation and analysis of the physical properties of skeletal muscle in terms of a force-generator system composed of viscous and elastic elements. Specifically, we estimated stiffness, believed to reflect myofilament cross-bridge activity, at various times during the contractile cycle and other quick stretch and release maneuvers. Also, we have estimated the level of activation of muscles exposed to two drugs known to potentiate the twitch response.

Methods Employed: We have applied techniques used in the engineering and physical sciences to investigate the physical properties of skeletal muscle. Stiffness (E) was calculated from measured values of the conduction velocity (C) of acoustic impulses, in resting or active muscle, which was applied to the relation:

$$E = C^2\rho,$$

where ρ is the density of the conductile element. The velocity measurement was relatively instantaneous i.e., made in a fraction of a m/sec which per-

mitted moment to moment assessment of muscle activity during various contractile maneuvers. The other major methodology employed was computerized mathematical modeling of these physical properties by Dr. Schoenberg with respect to known visco-elastic systems composed of Voit and/or Maxwell elements.

Major Findings: The acoustic impulses which we observed in skeletal muscle and inanimate visco-elastic systems were conducted by elastic elements and appeared to be independent of the influence of parallel viscous elements. This finding supports the interpretation that cross-bridge activity is reflected in the stiffness of the elastic elements which conduct these impulses. We also found that cross-bridge activity preceded but paralleled the development of active tension during isometric tetanic contraction in amphibian muscle. Another major finding demonstrated that "stiffness", assumed here to occur concomitant with the "active state" reached full intensity before either twitch or tetanic tension. These observations not only actually demonstrated this former hypothesis but permitted as well, an estimation of the rate of rise of the "active state". In addition, frog ringer solution containing NO_3 ion, a known potentiator of twitch response, did not increase cross-bridge activity; or restated, did not increase the intensity of the "active state". Likewise, frog muscle in the state of rigor, produced by long term stimulation in the presence of caffein, showed no increase in cross-bridge activity above normal isometric tetanic contraction. These latter observations confirm previous reports that these drugs which act directly on the contractile component do not increase its level of activity but merely extend the duration of activity.

Significance to Bio-Medical Research and the Program of the Institute: This work, in which physical techniques were used to evaluate particular physical processes in muscular contraction represents a fundamental approach to the study of active muscle. Although the current application of such information to clinical problems is at most extremely limited, nevertheless it is a necessary part of the general investigation of contractile mechanisms in skeletal muscle.

Proposed Course of the Project: Continuation of the project is aimed toward further identification of the particular components which carry the propagated impulses and their role in the current scheme of muscular contraction. It is important to repeat these observations on preparations of isolated single muscle fibers. Extraneous compliance and extracellular influences are largely eliminated in (this) the single fiber preparation, thus, an immediate effort is proposed toward acquisition of the necessary equipment and environment.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1050(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Functional Activity and Properties of Striated Muscles

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: I continued in the general objective of this project to observe and interpret changes in the contractile properties and responses of active skeletal muscle. Specifically, experiments were conducted to answer the question: does the heat which is liberated from muscle immediately after stimulation precede the mechanical changes which accompany activation? If positive, the answer implies that energy expended during contraction is mobilized from chemical sources as a result of stimulation, while if negative, it implies that resting muscle is analogous to a cocked spring which the stimulus releases. For the last several decades the first hypothesis has been the working model due mainly to the heat and mechanical measurements of A. V. Hill and his interpretation of the thermodynamic implications. However, his most recent of several reinvestigations of the first appearance of mechanical change after stimulation (nearly 3 decades ago) did not resolve the question. The development of apparatus and techniques in this laboratory for production and recording of very rapid, minute mechanical perturbations in isolated muscle has offered a fresh approach to this problem.

Methods Employed: Small discrete stretches, 0.25 mm in amplitude and 1 msecond duration were applied to one end of an isolated amphibian muscle at various times after electrical stimulation. Tension spikes produced by the stretches were recorded from the other end and observed to increase in amplitude during the stiffness change which normally accompanies the

spread of mechanical activation in muscle. The time after the stimulus when the evoked tension spikes just exceeded those recorded from resting muscle, was taken as the start of mechanical activation.

Major Findings: Initial observations suggest that elastic rather than viscous elements transmit the stretches in both resting and activated muscles. Also, mechanical activity began about halfway through the mechanical latent period which, according to Hill, is after heat liberation is well underway.

Significance to Bio-Medical Research and the Program of the Institute: Because the mere classification of human muscle diseases into etiologic subgroups is speculative at this time, an increase in knowledge at all levels of research is indicated. The certainty of these measurements compared to those of past investigations should provide the basis for correct conclusions concerning this particular point in the biophysics of muscular contraction. As part of the overall view therefore, knowledge concerning the functional mechanical components of contracting muscle will enhance the clinicians' ability to assess the pathophysiology of diseased muscle.

Proposed Course of the Project: I plan to extend these observations into both toad and tortoise muscle since these muscles are much slower and allow a better resolution in time of these rapid events.

Honors and Awards: None

Publications:

Goldstein, Seth R., Friauf, Walter S., and Wells, J.B.: A novel instrument for dynamic and static measurement of large length changes in muscle. J. Appl. Physiol. 36: 128-130, 1974.

Serial No. NDS(I)-67 MN/AP 1416(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.
Ahmed Hassan Mohamed, Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To obtain and investigate heretofore unavailable venomous substances. To determine the primary point of biologic activity and to explain the tissue and cellular mechanisms of these neuro-active substances. To fractionate the whole venoms into their active components.

Methods Employed: The above objectives require the use of a wide variety of whole animals, isolated tissue, and cellular research techniques involving toxicological, biochemical and biophysical techniques. Well known venoms are examined in order to establish a standard. A comparison of the standard with lesser known venoms facilitates the discovery of new properties of the various venoms with a minimum of search. Gel electrophoresis and column chromatography are used for venom fractionation.

Major Findings: Biological activity of venoms catagorized as neurotoxic has been found to progress with time and dose from initial paralysis of peripheral origin to cardiovascular toxicity, and eventually to CNS effects due indirectly to cardiovascular failure. Intra-arterially administered neurotoxic venoms damage the blood-brain barrier and permit entry of large dye molecules into the brain but intravenous administration of venoms is not followed by dye entry. This finding can be extrapolated to indicate that

snake-bite victims would probably not have CNS damage from neurotoxin, a point of some therapeutic interest. Additional venoms are now available from specimens collected from many points throughout Africa during the past year.

Significance to Bio-Medical Research and the Program of the Institute: Past research on venoms and naturally occurring toxins has been pre-eminently successful in various aspects of biology.

The collection of venoms from poisonous species has made possible the scientific development of antivenins for the treatment of envenomation by poisonous animals. The research here is collaborative with and supportive of a program that is now designed to collect venoms from the entire African Continent (except for the Union of South Africa) and to develop polyvalent antivenom for therapeutic use.

Because of the accelerating toxicity to which modern man is exposed, the study of toxic and poisonous substances becomes of increasing importance. Only a few relatively common venoms have been investigated. Over 600 different species of poisonous snakes are believed to exist in the world. What wealth of biological tools are undiscovered in the venom of the lesser known species can only be surmized from the success of past research. The relationships among snake venom neurotoxins, enzymes, antivenins, toxicities, and therapeutics are almost completely unknown. The project, therefore, has excellent possibilities of increasing progress in medical research.

The recent finding that snake venoms contain substances that function as nerve growth factors make this project especially relevent to the program of the Institute. The most recent example of the value of this type of project to science is the use of bungarotoxin obtained from the krait snakes and made radioactive to identify and locate the acetylcholine receptor on skeletal muscle fibers.

Proposed Course of Project: The termination date of the project has been delayed for an additional year without additional funding to allow a further examination of the needs of the Institute for a continuation. If a continuing Institute interest is found, a further project extension can be sought. The project has been inactive this year because the Principal Investigator is on detail as the Assistant Director of the Intermural programs.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-70 MN/AP 1809(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Impaired Transfer of Information Between Nerve and Muscle

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B.S.

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To find biochemical deficiencies that impair transfer of biological information between nerve and muscle.

Methods Employed: Suitable animals are made deficient in a component of a metabolic pathway suspected of being required for transfer of information between nerve and muscle. Efficiency of information transfer is studied *in vivo*. Restoration of function is determined by return of metabolic substance or a precursor to the animal during testing. Simultaneous comparisons are made between muscle types.

Major Findings: Last year it was stated that adult rats kept without dietary choline and allowed access to their total environment practice coprophagy, recycle choline and transfer information normally between nerve and muscle. Weanling rats denied choline became metabolically deficient as indicated by increased number of deaths, loss of hair, eye and kidney hemorrhage, cataracts, large fatty livers and slow growth rates. These severely choline deficient rats maintain a near-normal ability to transmit signals between nerve and muscle. This indicates that under severe choline depletion the nervous system can utilize or synthesize choline to a greater degree than certain other tissues or that motor nerves may have a low choline requirement. It is of general interest to the neurological sciences that animalswith major defects in many organ systems because of lack of dietary choline have no apparent functional deficiency in the efferent motor nerves.

Weaning rats made methionine deficient by dietary controls fail to gain weight but do not exhibit the physiological symptoms of choline deficiency or a transmission deficit between nerve and muscle. Animals made both choline and methionine deficient likewise fail to develop a defect in transfer of information from nerve to muscle. These later findings can be interpreted to mean that alternate pathways can supply one-carbon fragments or methyl groups in sufficient quantity to synthesize sufficient choline and subsequently enough acetylcholine to maintain transmission of information between nerve and muscle. The supplying of methyl groups by synthesis through one-carbon metabolic pathways would function as a protective mechanism for the nervous system against dietary deficiency of either choline or methionine. The functioning of such a protective system would have heuristic implications for organism survival since the nervous system, the primary organ for integrative functions, would be preferentially protected from dietary insufficiency of a chemical necessary for normal function. This year the project has been inactive because the Principal Investigator is on detail as the Assistant Director of the Intramural program.

Significance to Bio-Medical Research and the Program of the Institute: There is great need for new knowledge about the processes involved when muscle fails to function in neuromuscular disorders. There is incomplete information about the biochemical substances that maintain the functional and structural integrity of muscle in the normal state. Identification of metabolic chemicals that are necessary for transfer of information from nerve to muscle may aid in determining the pathogenesis of some of the neuromuscular disorders, a useful step in designing of therapeutic measures.

Proposed Course of Project: This project should be continued with an emphasis on identification of the biochemical substances that are necessary to maintain the functional integrity of skeletal muscle. The future of this project has not been defined since the Principal Investigator is on detail as the Assistant Director of the Intramural program.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Surgical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

J. M. Van Buren, Chief

Summary of Studies in Surgical Neurology:

The primary orientation of the Branch is to study the form, function and disease of the human nervous system as relevant to neurological surgery. Long-term projects deal with studies in the following categories: I. Dyskinesias; II. Epilepsy; III. Speech, Memory and Cognitive Function; IV. Trauma of the CNS; V. Tumors; and VI. Vascular Disease of the CNS. A pilot study is being made in Pain.

I. Dyskinesias (Stereotaxic Program)

Clinical material in which depth electrodes were used have provided patient material for study of speech, memory and cognitive function.

II. Epilepsy (Surgical Epileptic Program)

Basic Studies:

A. Review of the alumina cream macaque epileptic model has reemphasized the difficulties of obtaining a "mirror focus". Studies are planned to utilize this material in physiological evaluation of the inhibitory surround and of the epileptic focus and an anatomical study of the morphology of epileptic foci in propagating pathways through histological techniques demonstrating significant stages in epileptic metabolism.

B. Studies of synaptic function and morphology are being undertaken in the 3-mercaptopropionic acid rat epileptic model and in human operative epileptic samples.

C. Basic studies carried out by N-EEG have indicated that in the penicillin model at least fluorometric NADH fluorometric recording will differentiate between the primary and projected epileptic activity. Technology is being pressed to make this apparatus available for real time use in the operating room.

D. In collaboration with N-LNLC a technique is now available which promises to provide stable recordings of extracellular cortical activity under operating room conditions. This offers an opportunity to further evaluate the chronic human epileptic focus.

Clinical Studies:

A. The equipment is now available for current controlled transcutaneous stimulation of the human cerebellum in the control of chronic, intractable

cerebral seizures in man. A pilot study of five cases of such implants is being initiated.

B. In collaboration with N-EEG the late follow-up results of 143 temporal operations for epilepsy have been evaluated at 3-18 years follow-up. The need for the long period of follow-up is strongly emphasized as well as the demonstration of a number of criteria which bear upon the surgical technique in the prognosis.

The special study of patients with combined bitemporal and temporal extratemporal foci demonstrated a reasonably hopeful outlook for these patients with temporal lobectomy which had not previously been suspected.

III. Speech, Memory and Cognitive Function (Surgical Epileptic and Stereotaxic Program)

A. Research is concerned with neurophysiological and psychological changes following amygdaloid and cingulate lesions in man. Computer programs are currently being developed to assess the effects of surgery in changing the pain threshold of patients and to evaluate the effects of limbic lesions on personality and emotional status.

B. In the studies of patients undergoing electrical stimulation of the exposed brain in the course of surgery for the relief of epilepsy, it was possible to map in the posterior temporoparietal regions of the left hemisphere distinct areas which support the ability to identify pictorial verbal material. Stimulation of the anterior cortical areas of the left temporal lobe and a wide area of the right brain failed to disrupt naming skills. Memory presses the anterior and posterior temporal regions into different support services. The primary or immediate memory system may be initially linked with the anterior temporal lobe and medial structures (hippocampal complex) while the posterior temporal parietal cortex may support secondary or long-term memory.

C. Perception and memory were studied during electrical stimulation with electrodes implanted in the lateral thalamus. Stimulation in the left pulvinar induced transient dysphasia and retrograde loss in recent memory for verbal memoranda. In contrast, comparable stimulation in the right pulvinar failed to disrupt verbal behavior and, instead, interfered with perception or recognition of complex visual patterns. Although the pulvinar nuclei behaved like related ipsilateral cortices with regard to general verbal or nonverbal functions, the cortical and subcortical stimulation results were different. Specifically, dysphasia and anmesia were indistinguishable effects during cortical but not during thalamic stimulation. Thus, the contribution of the pulvinar and other posterior thalamic nuclei may be limited to pre-perceptual processing and may serve to shuttle sensory information to the cortical stations for final interpretation and analysis.

D. Collaborative studies with the Aphasia Unit in the Boston VA Hospital on comparing behavioral deficits in the patients with Korsakoff's

syndrome, parietal or temporal lobe lesions suggest that the cortical lesions in the left hemisphere affect learning or auditory information, while those on the right influence visual learning.

IV. Trauma of the CNS (No In-House Clinical Program)

Basic Studies:

A. The crucial role of rotational loading for diffuse lesion production and of translational and contact phenomena components of impact for focal lesion production first demonstrated in the squirrel monkey are being confirmed and in the larger rhesus monkey brain permitting a closer analogy to man. Study of the effect of dynamic mechanical loading of frog sciatic nerve has shown a direct correlation between load intensity and amplitude of the compound action potential to compressive-tensile loads. The animal models are being used to develop trials of protective and therapeutic modalities for the amelioration of head injury severity.

Clinical Studies:

(Collaborative Study at D. C. General Hospital)

A. Severely injured patients with absent short latency waves in the visual and somatosensory evoked potentials on initial examination never recover full neurological function if these components show no recovery. It has been found that when short latency waves recover but subsequently disappear in a severely injured patient, a progressive and often surgically remedial lesion was found. Objective changes in a neurophysiologic assay including evoked potential data have been observed which correlate significantly with the symptoms of the posttraumatic syndrome.

B. In angiographic studies of spinal cord injuries, the sulco-commissural arteries have been demonstrated by high magnification in one patient. Extravasation of contrast medium into the epidural space has been shown in one case, indicating bleeding in progress. One of the tetraplegic patients who had been studied by angiography later died and it was possible to carry out a postmortem microangiographic study of the spinal cord. Thus it was possible to compare in vivo angiography and postmortem microangiography in the same specimen.

C. A predictive algorithm for head injury severity in man, based on our animal models, is being developed for use at the D. C. General Hospital.

V. Tumors of the CNS (Clinical Tumor Program)

Basic Studies:

A. The animal model has shown that preimmunization and chemotherapy are effective ways of treating the intracerebral murine tumor, but immunotherapy alone is a totally ineffective form of treatment. The

survival of animals treated with immuno-chemotherapy was not significantly prolonged over that of animals treated with chemotherapy alone, although a reduction in the dose of chemotherapy required was suggested.

B. Histologically verified intracerebral delayed hypersensitivity reactions could be evoked in immune nontumor bearing primates with no significant neurological side effects when intracerebral PPD was given to PPD skin-test positive animals. Increased phytohemagglutinin responsiveness of the animals lymphocytes was also demonstrated in vitro suggesting that this enhancement of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumor immunotherapy. Further enhancement of PHA responsiveness was also demonstrated to nitrogen mustard and dinitrochlorobenzene, suggesting that such agents also may have adjunctive therapeutic value.

C. Studies continue to characterize the cell surface and to isolate the carcino fetal antigen which if successful would support immunotherapy as a powerful therapeutic tool.

Clinical Studies:

A. Diagnostic methods:

1. Cisternography: A study of normal CSF flow patterns with emphasis on the spinal CSF descent has been continued in the rhesus monkey and in man. The initial experience that failure of flow over the hemispheres may coincide with the location of an intracerebral tumor has been confirmed. There has been further experience in assessing the progression of neoplastic ventricular invasion, distribution of intrathecal medication and CSF shunts.

2. Work has been continued on the Tomoscanner with changes in the geometry of the detecting probe and an indexer for tape records. These changes have improved both the resolution of the instrument and the ease of use. An extended clinical trial is planned.

3. Initial experience is being gained with computerized axial tomography for brain tumors as well as of the hindbrain and spinal cord. In one instance it has been possible to resolve the central cavity in syringomyelia.

B. Clinical studies:

1. Two patients with malignant glioma treated with immunotherapy alone had recurrences of their tumor before patients receiving chemotherapy or immuno-chemotherapy.

2. A majority of glioma patient's lymphocytes are less effective in killing their own tumor cells than are lymphocytes of normal controls, irrespective of whether chemotherapy or immuno-chemotherapy is given after initial surgery and radiation therapy.

3. Histologically, brain tumor biopsies from patients undergoing immunotherapy or immuno-chemotherapy, reveal lymphocytic infiltration of the leptomeninges and subpial areas, but no significant increase in numbers of lymphocytes within the substance of the brain tumors can be claimed because of the small amount of data and sampling errors due to biopsy limitations.

4. To date, the combined chemotherapy regimen using oral CCNU and intratumoral 8-Azaguanine appears to offer the best improval survival data (> 24 months for 50% survival as compared to < 12 months for untreated controls).

VI. Vascular Disease of the CNS (No In-House Clinical Program)

A. Basic Studies

1. A correlative study between sequential radionuclide brain scanning and time lapse microangiograms has been completed. In the majority of animals, brain scintigraphy became positve by 2 weeks to regress toward negative by 4-6 weeks after ligation of the middle cerebral artery. The increased isotope uptake in the affected area was clearly related to neovascularization around the area of the infarct as shown by the microangiogram. Decreased vascularity, peripheral gliosis and central cavity formation are the main factors determining the diminution of the radionuclide penetration in the involved area at later stages.

B. Diagnostic Methods

1. Following clinical observation of postangiographic spinal cord damage, a primate model with a definite threshold of production of postangiographic paraplegia is being established. This model will be useful in testing various types of new contrast media. Evoked spinal cord potentials will be used to determine the point of early cord damage. In addition, an EM study will be made of the damage to the vascular tight junctions.

VII. Acupuncture

Study of the first eight normal controls in an attempt to determine whether acupuncture produces elevation of pain threshold so far has produced negative results.

Branch Orientation and Planning:

No change is contemplated in the general character of the patient disease categories under study or the emphasis upon the study of the human nervous system function and disease. The epilepsy and brain tumor programs have been expanded by the addition of a neurochemist.

Serial No. NDS(I)-54 SN/OC 100(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M.D., C. Ajmone-Marsan, M.D. and A. Daniel, Ph.D.

Other Investigators: A. K. Ommaya, M.D., F.R.C.S., P. Fedio, Ph.D., D. Lewis, M.D. and N. Mutsuga, M.D.

Cooperating Units: N-EEG and N-LNLC

Man Years

Total:	1.6
Professional:	1.2
Other:	0.4

Project Description:

Objectives:

1. To study causal mechanisms of epileptic seizures in man and other primates.

2. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.

3. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

4. To make use of opportunities in diagnosis and therapy for the study of neurophysiological and neuropsychological problems.

Methods Employed:

1. Clinical neurological examination.

2. Special radiographic and other contrast examinations.

3. Electrographic, including electrocorticographic, examination.

4. Physiological and psychological techniques as indicated.

5. Histological and chemical examinations as required.

Major Findings:

1. Basic Studies in Epilepsy

a) Chronic cortical lesions of the alumina cream have been prepared in 21 macaque monkeys between 6 months and 1 year ago. Implanted recording devices have been placed to facilitate reproducible recording conditions. To date none of the hoped for "mirror foci" have appeared in the monkey and it appears that these are not practical subjects for a study of this phenomenon.

b) The rat model, using the convulsant 3-mercaptopropionic acid, is being investigated. The brains of repeatedly convulsed animals, once convulsed animals and control animals are being collected and the synaptic membranes isolated for specific study. This has proven to be a consistent dose related model.

Synaptic membranes obtained from human epileptic foci have failed on initial studies to show a significant change between these membranes and the "normal" membranes obtained in the same patient from nonepileptic areas. To further investigate this matter, a method was developed using radioactive plant lectins to investigate the presence of macromolecular surface carbohydrates.

2. Clinical Studies

a) The late follow-ups of 143 temporal lobe epileptics has been finished and this material is summarized in Project #NDS(I)-72 EEG/CN 1940(c).

b) A special study has been made of a group of temporal epileptics with bilateral and/or extratemporal foci in terms of the late surgical results.

In the epileptic group characterized by automatisms (psychomotor seizures) with temporal epileptiform activity, but complicated by EEG foci in the opposite temporal lobe or extratemporal activity, temporal lobectomy following careful and repeated EEG evaluation (with the use of implanted electrodes in otherwise unresolvable cases) can result in rendering a significant number of patients (between 25-50%) either seizure free or with significant and useful reduction in their seizure frequency.

In 28/34 of these patients with postoperative epileptiform activity on the EEG, 2/28 had the appearance of new epileptiform activity in the opposite temporal lobe, and 1/28 had activity appear elsewhere during the follow-up period of 3-17 years (av. 8 yrs.).

b) Since 3-mercaptopropionic acid in vitro is an inhibitor of glutamic acid decarboxylase, it is planned that a neurochemist who will be joining us in September will look into the biosynthesis and degradation of GABA in vivo and in vitro. The synaptosomal membranes will be studied with radioactive lectins for evaluation of the surface glycoproteins and the membrane Na-k ATP'ase activity. Collaborative EM study of the synaptosomal membranes is planned with the Laboratory of Neuropathology and Neuroanatomical Sciences. Study of the carbohydrate portion of the glycoprotein and glycolipid fractions of synaptic membrane preparations "normal" and epileptic human cortical foci will be pursued on a collaborative basis with Dr. R. W. Wheat (Duke University Medical Center). Concomitant study of the rat model will be undertaken.

2. Clinical Studies

a) Follow-up results on surgical treatment of extratemporal foci will be undertaken along the lines worked out in the initial study on temporal lobe seizures.

b) The NADH fluorometric monitoring remains as yet a laboratory tool due to the lack of the availability of real time analysis. Instrumentation for color conversion of minor black-white differences on the TV image has been looked into and is technically feasible. Instrumentation, however, has been temporarily held up. In the experimental model it has been shown that projected spikes do not produce the reduction in NADH fluorescence seen with primary cortical foci (penicillin). This offers some possibility of making this distinction in the human electrocorticogram which to this time has posed an insoluable problem.

c) Development of a microelectrode supported on an air column by the N-LNLC has provided, in animals at least, a microelectrode which rides freely with the cortex and can maintain satisfactory and long-lasting relationships with cortical neurones despite the cortical pulsations. This electrode will be tried in human cases. The preliminary study will be the unit firing concomitant of spontaneous epileptic spikes in the human cortex, Project # NDS(I)-69 LNLC/OC 1687.

d) In collaboration with the N-LNLC, the fabrication of units permitting transcutaneous stimulation through RF coupling with current control has been accomplished. The first of five patients in the pilot study of the inhibitory effects of chronic cerebellar stimulation on intractable cerebellar seizures will have the device implanted in May 1974.

e) The proposed project on the effect of H-field lesions on intractable epilepsy which was proposed and carried to the Clinical Research Committee, NIH in the spring of 1973 has as yet not been acted upon by this committee.

The results suggested that complete mesial temporal removal during temporal lobectomy increased the frequency of cure.

Seizure remission up to 15 years with eventual recurrence of the original seizure type may occur following surgical therapy.

Follow-up studies of surgical epileptic treatment of less than 3-5 years are of doubtful value.

c) Fluorometric NADH recording continues to be carried out during open craniotomy as the occasion arises. Satisfactory records at different time-current levels of stimulation have been obtained in eight patients. Although the product of stimulation current and time is related to the reduction in NADH fluorescence, the slopes of these curves has proved so far an individual characteristic. However, in this pilot study too few data points per patient were obtained. It is hoped that this data can be refined now that the recording method has become more routine. The majority of the intra-mitochondrial fluorescence obtained from cortex is within the upper 0.5 mm. An interesting case of cortical dysplasia in which the marginal layer contained neurones showed an apparently "normal" fluorometric response to stimulation despite the gross gliosis and distortion of the cortical layers. The Golgi morphology of these remaining neurones, however, appeared normal. Thus, it is hypothecated that in this unique case a displacement of neurones toward the pial surface where they were more accessible to fluorometric monitoring compensated for the reduced signal which would have accompanied the gross depopulation of the cerebral cortex. This is an interesting sidelight which would have to be interpreted in the future interpretation of these records. (For further basic details see the Project # NDS(I) - 72 EEG/CN 1939b of the Branch of Electroencephalography and Clinical Neurophysiology.)

d) Patients with chronic implanted depth electrodes and undergoing craniotomy under local anesthesia with cortical stimulation have provided material for Project # NDS(I)-68 SN/OC 1658(c).

Proposed Course of the Project:

1. Basic Studies

a) In the coming year the chronic alumina cream epileptic lesions will be subjected to electrophysiological studies with particular attention to the inhibitory surround about the epileptogenic areas and will be subject to special staining procedures. It is hoped to set up a collaborative effort in this regard with Dr. E. Roberts, whose team has developed a method for visualizing glutamic acid decarboxylase. A Visiting Scientist, shortly to join us, will spend full time on this project.

Significance to Biomedical Research and Program of the Institute:

1. The potential capability of histologically marking a significant episode in the metabolic cycle of the epileptic neurone makes theoretically practical the study of the propagation of epileptic activity as an anatomical rather than a physiological matter. The question remains whether this can be translated into a practical methodology which could then benefit from our previous experience with the neuroanatomy of the primate brain.

2. The multifaceted approach to study of synaptic activity in the animal model and the human epileptic brain may provide important insights into the basic mechanisms of epilepsy.

3. Techniques now appear available for nearly immediate application which may provide information to differentiate primary versus projected spikes on the ECoG and reliably document unit activity in the spontaneous clinical EEG focus at human craniotomy.

4. One of the important features arising from the late follow-up studies of epilepsy has been the necessity for a minimum of 3 years of postoperative follow-up before attempting to analyze the results. The majority of results cited in the literature thus appear to be invalid due to neglect of this single criterion.

Honors and Awards: None

Publications: Van Buren, J.M., Ajmone-Marsan, C., and Mutsuga, N.: Temporal lobe seizures with additional foci treated by resection. J. Neurosurg. In press.

Serial No. NDS(I)-55 SN/OC 200(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Involuntary Movements

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M.D. and P. Fedio, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 1.5
Professional: 1.1
Other: 0.4

Project Description:

Objectives: Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed: Depth electrode stimulation employs specially built electrodes and a current-monitored stimulator. Special apparatus used in quantitative psychological testing is described in Dr. Fedio's project report Serial No. NDS(I)-68 SN/OC 1658(c).

Major Findings: Continued study of the Arrest Response has confirmed and extended earlier observations indicating:

1. The spatial separation of the arrest response (striatal area) from sensori-motor and speech modification affected by subcortical parietal and thalamic stimulation.

2. Frontostriatal stimulation resulting in speech interruption has, without exception, been accompanied by total amnesia for the presentation. In other words, the patient has been unable to either respond or store the visual stimulus during the stimulation.

3. Testing of speech function with striatal stimulation (in contrast to thalamic and parietal stimulation) showed that in the former instance the response was an all or none matter while the latter could show a response with graded latency indicating storage and the ability for later recovery.

4. Study of material provided by the depth electrode implantation has been continued by the Neuropsychologist. (Project # NDS(I)-66 SN/OC 1245(c), "EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes"; NDS(I)-67 SN/OC 1424(c), "Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions"; and NDS(I)-68 SN/OC 1658(c), "Hemispheric Development and Specialization of Intellectual Functions.")

Significance to Bio-Medical Research and the Program of the Institute:

The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible even with the time consuming conditioning experiments applied in lower animals. The use of the human subject is therefore not a duplication of animal experimentation but an extension of this; and, of course, studies of speech function may only be carried out in man.

Proposed Course of the Project:

Dyskinesia patients will be studied as they become available in support of projects related to neuropsychological studies. At the present time, the personnel support does not permit further studies utilizing the neurophysiological opportunities offered by the surgical approach.

Honors and Awards: President, American Society of Stereotaxic and Functional Neurosurgery

Publications: None

Serial No. NDS(I)-56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Previous Serial Number: Same

Principal Investigator: J. M. Van Buren, M.D.

Other Investigators: R. C. Borke, M. G. Levine, M.A. and F. P. Wirth, M.D.

Cooperating Units: None

Man Years

Total:	2.4
Professional:	0.3
Other:	2.1

Project Description:

Objectives:

This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

1. Serial sections of human and animal brains in celloidin for myelin and Nissl series.

2. Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

1. Although the earlier supposition was that the n. dorsalis superficialis (n. lateralis dorsalis) of the thalamus projected to the parietal region, more recent evidence has linked it to the posterior cingulate gyrus and possibly adjacent regions near the splenium of the corpus callosum. An afferent supply from lower levels was in more doubt, although some report had been made of cell and fibre degeneration in the n. dorsalis superficialis

after extensive temporal resections and section of the fornix in lower primates. The five human hemispheres of the present study all had lesions of long duration below the level of the splenium of the corpus callosum in the posteromedial temporal region. All showed marked degeneration in the fornix and n. dorsalis superficialis. In favourably stained cases, gliotic fascicles could be followed from the descending column of the fornix to the n. dorsalis superficialis via the region lateral to the stria medullaris thalami. The cell loss in the nucleus thus appeared to be an instance of anterograde transynaptic degeneration. These cases provided an interesting instance in which human infarctions provided natural lesions that would have been hard to duplicate in experimental animals.

2. Studies of the topographical relationship between the site of cerebellar inflow of the thalamus and the thalamo-precentral projection in the chimpanzee.

Following section of the superior cerebellar peduncle in two animals (C-I-64; C-I-65), degeneration demonstrated by the Nauta-Gygax method entered the anterior portion of the large-celled nucleus called VP in the Walker (1938) nomenclature but separated by others as the n. ventro intermedius (Vogts, 1941; Hassler, 1959; Van Buren and Borke, 1972) or VPLo (Olszewski, 1952). Degeneration in the medial lemniscus following the lesion of the n. fasciculus gracilis (C-I-67) enters the large-cell nucleus (VP) immediately posterior to the cerebellar inflow or the n. ventro caudalis (Van Buren and Borke, 1972).

Precentral ablation (C-I-65). In a precentral ablation limited to the face and leg regions demonstrated by cortical stimulation (C-I-69) cell degeneration in 60-90 days was limited to the lateral ventral thalamic region between the mamillo thalamic tract and the large cell (VP) nuclei or VL of Walker. This region receives the ramifications of the cerebellar inflow funneling in through the n. ventro intermedius. When face and leg areas were ablated selectively, the areas of retrograde change lay respectively in the anteromedial and posterosuperior lateral areas suggesting a thalamic representation of the precentral gyrus that runs obliquely to the conventional transverse axis of the thalamus.

These studies in the chimpanzee which have been supported in part by human studies thus indicate that the traditional studies suggesting that the cerebellar inflow entered (VL) initially are in error, since the initial entry is in the anterior portion of the large cell nucleus just behind it (ventralis intermedius). This mistake is understandable inasmuch as the Marchi method available at the time did not permit close monitoring of the cytoarchitectural fields. However, this error is in all of the current textbooks. Likewise, the diagonal thalamic representation of the precentral gyrus in the lateral ventral thalamus is entirely against the many textbook illustrations demonstrating a strict medial-lateral arrangement. It is evident that rechecking of the traditionally accepted anatomy which is

supplied from studies in lower animals must be carried out with studies in high primates and man if these findings are indeed to apply to the work of the clinical neurosciences.

Proposed Course of Project:

The availability of new techniques for the study of enzyme histochemistry offers the exciting prospect of having the means to undertake topographical studies in high primates of metabolic processes and relate the relative activities to the cytoarchitectural entities of the thalamus, basal ganglia and remaining diencephalon and brain stem. Once these basic maps are made up, the technique can be applied to other studies such as that of the experimental epilepsy (see Project # NDS(I)-54 SN/OC 100(c)).

The problems, of course, center upon the specialized talent as well as time and space required to produce the "stains." Since we cannot hope to obtain this talent within the Branch, it is obvious that some type of collaborative work must be undertaken. We are fortunate in having access to Dr. Bloom at St. Elizabeth's Hospital and Dr. Kaufmann in Building 36 (who has a preparation to detect tyrosine hydroxylase). Particular interest has been paid to Dr. Roberts who can selectively label glutamic acid decarboxylase with perioxidase which would apply well to the epileptic project. The logistics of collaboration here are difficult since he resides in California. The possible use of a tritium label opens further possibilities for topographical studies. With the exception of one paper from Roberts group, no studies of this type were reported at the current meeting of the American Association of Anatomists, indicating our opportunity to advance rapidly in this field if proper liaison can be made.

Honors and Awards: None

Publications: Wirth, F.P.: Insular-diencephalic connections in the macaque. J. Comp. Neurol. 150: 361-392, 1973.

Van Buren, J.M., and Borke, R.C.: Nucleus dorsalis superficialis (lateralis dorsalis) of the thalamus and the limbic system in man. J. Neurol. Neurosurg. Psychiatry. In press.

Van Buren, J.M.: Proceedings of the 5th International Congress of Neurological Surgery, Tokyo, 1973. In press.

Serial No. NDS(I)-62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Trauma to the Nervous System.

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: S. Nakatani, M.D., T. A. Gennarelli, M.D., D. Carpenter, M.D., L. Thibault, M.S., B. Bhussri, Ph.D., J. Bossom, Ph.D., Norman Peter, M.D.

Cooperating Units: Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH; Departments of Anatomy and Surgery (Division of Neurosurgery), Georgetown University School of Medicine; Department of Neurobiology, Armed Forces Radiobiology Research Institute.

Man Years:

Total:	3.5
Professional:	2.5
Other:	1.0

Project Description:

Objectives:

1. To understand the mechanisms of mechanical trauma to the nervous system and its responses to grades of such trauma.
2. To design rational methods for protection, prevention, and treatment of trauma to the brain and spinal cord.

Methods:

1. Utilizing programmable head accelerating devices, sub-human primates are subjected to controlled head accelerations, separating the injurious effects of inertial loading and contact phenomena of impact.
2. Utilizing a programmable contusion device and set of implanted balloon probes, the effect of controlled brain distortions on the cerebral circulation and intracranial hydrodynamics at varying sites in the rhesus monkey is being determined.

3. Utilizing a PDP-12 computer, averaged evoked potentials are being tested as part of a neurophysiologic assay for primary injury severity and diagnosis of secondary brain lesions, both in our animal models and in head injured patients.

4. Utilizing simple neural preparations (initially frog sciatic nerve) to determine the effect of mechanical loading at various frequencies on neural functions and structure at the microscopic level (microtrauma project).

5. Utilize histologic techniques (light and electronmicroscopy and histochemistry) to seperate the primary and secondary damage to neural and vascular structures and to evaluate the role of the nerve supply to the microcirculation and the biogenic amines in the reactive changes to trauma.

6. A model to seperately evaluate the role of vasospasm and mechanical factors in cerebral ischemia after intracranial haemorhage is being developed.

Major Findings:

1. The crucial role of rotational loading for diffuse lesion production and of translational and contact phenomena components of impact for focal lesion production first demonstrated in the squirrel monkey are being confirmed and further analyzed in the rhesus monkey brain; the larger brain mass in this species allowing a closer analogy to the human brain.

2. Analysis of the neurophysiologic data from patients showed that:

a) Severely injured patients with absent short latency waves in the visual and somatosensory evoked potentials on initial examination never recover full neurological function. When short latency waves recover but subsequently disappear in a severely injured patient a progressive and often surgically remediable lesion was found.

b). Patients with symptoms of the post-traumatic syndrome (lethargy, headaches, memory disturbances, dizziness etc.) have evidence of abnormal signal processing in the neurophysiologic assay. These objective abnormalities seem to clear with time and are being studied as an objective tool for documenting injury severity and prognosis in patients after head injury.

3. Experiments with an extradural intracranial balloon in the monkey have shown that the measurement of intracranial pressure (ICP) and determination of critical perfusion pressure (systolic arterial pressure - intracranial pressure) are not useful indices of fluid/mass dynamics in neurologic disorders. The importance of the rate of volume changes has also been revealed by these experiments and a critical rate of mass expansion in the monkey below which a Cushing reflex is not elicited and the monkey suffers brain death at relatively low ICP has also been discovered. (< 0.02 cc/minute)

4. A unifying hypothesis for cerebral concussion and traumatic unconsciousness which is supported by and fits with all existing experimental data and clinical observations has been developed and is now being tested.

Significance to Bio-medical Research and the Program of the Institute:

1. Our current work has established reproducible models in sub-human primates for work on improving head injury management and analysis of post-traumatic sequelae in the rhesus monkey. These models are now being duplicated at the Head Injury Center of the University of Pennsylvania by Dr. T. Langfitt.

2. The studies in patients are being correlated with the animal studies. This will enable quantification of the input-response relationships in head injury. Our hypothesis for cerebral concussion predicts that the major part of post-traumatic deficit is due to the primary damage. If supported by our current work this will radically alter the therapeutic approach to patient care after head injury.

Proposed Course:

1. Validation of current and new models of head injury to develop a rational set of standards for diagnosis and treatment as well as prevention and protection against trauma to the head.

2. Develop long term studies of post-traumatic sequelae after controlled head injury in primates and head injury in man.

3. Evaluate our neurophysiologic assay as an index of head injury severity in man, with special reference to the in-vivo diagnosis of primary and secondary effects of trauma and their relative contributions to the genesis of post-traumatic sequelae.

Honors and Awards: 1. Nominated U.S. Representative to International Research Committee on Biokinetics of Impact.

Publications:

Gennarelli, T.A. and Ommaya, A.K.: A Neurophysiologic Assay of Head Injury Severity. Experimental anc Clinical Observations. J. Neurosurg. (in press)

Nakatani, S. and Ommaya, A.K.: The Intracranial Pressure - Volume Relationship in the Rhesus Monkey. J. Neurosurg. (in press)

Ommaya, A.K.: Head Injury Mechanisms. Final Report on Work Conducted under Inter-Agency Agreement No. DOT-HS-081-106- IA between National Highway Traffic Safety Administration and National Institute of Neurological Diseases and Stroke. Sept. 1973. National Technical Information Service, Springfield, Virginia.

Ommaya, A.K. and Gennarelli, T.A.: Experimental Head Injury. Handbook of Neurology, Volume 22/23. Edited by P.J. Vinken and G.W. Bruyn. (in press)

Ommaya, A.K. and Gennarelli, T.A.: Neural trauma: correlations between the biomechanics and pathophysiology of head injury. Proceedings of the Symposia of the Fifth International Congress of Neurological Surgery, Tokyo, October 7-13, 1973 (in press)

Serial No. NDS(I)-62 SN/OC 913(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objective: To study the activity of single nerve cells in the cerebral cortex and subcortical nuclei in relation to motor and sensory functions.

Methods Employed: Two micro-electrodes are placed, one in the motor cortex and the other in the thalamus of patients undergoing operative procedures. The other parameters, e.g. EEG, EMG, are also measured in response to motor movement or sensory stimulation.

Major Findings: Some cells in the motor cortex were found to be active in close relation to motor movement, such as Parkinson tremors, voluntary flexors or extension of the opposite extremity. A few showed suggestive evidence that the activity is dependent upon the activity of the thalamic neurons.

Significance to Biomedical Research and the Program of the Institute: The program will add to our knowledge of normal and abnormal functions of the motor system in man.

Proposed Course of Project: Multiple microelectrodes implanted in various structures of the striatum to record simultaneously the activity of the cells for long periods and to study the correlation of this activity and

the specific motor movement of the patient will be a great leap forward in this line of research.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Tumors of the Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, M.D., F.R.C.S., F.A.C.S.

Other Investigators: Leland Albright, M.D., W. Terry, M.D., Amiram Daniel, Ph.D., John Madigan, M.D., Max Cohen, M.D., Ph.D., Leslie Cahan, M.D.

Cooperating Units: Immunology Branch and Surgery Branch, Division of Cancer Biology and Diagnosis, National Cancer Institute.

Man Years:

Total:	3.0
Professional:	2.5
Other:	0.5

Project Description:

Objectives:

1. To develop immunochemotherapeutic methods for the treatment of human malignant gliomas.
2. To evaluate immunologic parameters of patients undergoing therapy of brain tumors.
3. To develop animal models suitable for evaluation of immunotherapy and chemotherapy trials.
4. To evaluate the:
   a) immunological identification of tumor specific antigens.
   b) chemical make-up of tumor-associated antigens, initially assumed to be in glycoproteins isolated from tumor cell membranes.
   c) Antigenicity of tumor associated glycoproteins.

Methods Employed:

1. Patients with histologically verified glioblastoma multiforme and malignant astrocytomas (Grades III and IV) are selected. The extent of neurological deficit and intracranial mass anatomy is established clinically and neuroradiologically, including evaluation by computerized axial tomography before and after maximal tumor resection and treatment with radiotherapy (conventional or fast-neutron therapy at the NRL Cyclotron).

2. Cerebrospinal fluid reservoirs and intratumoral cysts are inserted to allow evacuation of tumor bed contents and for infusion of chemotherapeutic agents or agents to induce intratumoral delayed hypersensitivity reactions.

3. Patients are randomized into a prospective controlled study to evaluate combined chemotherapy alone versus similar chemotherapy combined with immunotherapy.

4. The program of combined chemotherapy utilizes oral CCNU 130 mgm/sq. meter body surface and intratumoral 8-Azaguanine 100 mgm by infusion; the oral drug being given for six-doses, one dose per 6-8 week period or until onset of liver or marrow disturbance. The intratumoral drug is given once a week for six weeks and then once a month for one year and once a month indefinitely after that.

5. The immunotherapy consists of immunization with BCG followed by intratumoral PPD to elicit a delayed hypersensitivity reaction in the residual glioma. In addition the patient's tumor cells are given subcutaneously after pre-treatment with mitomycin and neuraminidase. In patients receiving both chemotherapy and immunotherapy the two modes of therapy are given at alternate intervals.

6. Cytotoxicity (^{51}Cr) assays are used to compare the ability of normal lymphocytes and brain tumor patients' lymphocytes to kill brain tumor cells.

7. A murine glioma model has been grown in tissue culture to provide a tumor model which can reliably induce intracerebral tumors in mice and provide large numbers of cells for immunotherapy of that tumor.

8. Rhesus monkeys have been immunized with BCG, then injected intracerebrally with PPD to evaluate the nature and extent of intracerebral delayed hypersensitivity reactions in an animal model which is immunologically related to man.

9. A tissue culture method which will yield a large number of glioma cells is currently being evaluated.

10. As a preliminary trial, a large amount of cell membrane from the murine glioma cells have been isolated. The glycoproteins of these cell membranes are being solubilized by the lithium disodosalicylate procedure. These glycoproteins are separated by a polyacrylamode gel electrophoresis and the fractions are used to raise, in rabbits, a relatively pure population of anti-glioma antibodies. A similar approach will be followed in larger glioma samples obtained from fresh autopsies in patients dying with this tumor.

11. Methods to isolate and identify "blocking factors" in serum from tumor bearing hosts are being developed.

12. The technique of computerized axial tomography is being tested 'in-vivo' with patients and 'in-vitro' using phantoms.

Major Findings:

1. Histologically verified intracerebral delayed hypersensitivity reactions could be evoked in immune nontumor-bearing primates with no significant neurological side effects when intracerebral PPD was given to PPD skin test positive animals. Increased PHA responsiveness of the animals' lymphocytes was also demonstrated in-vitro suggesting that this enhancement

of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumor immunotherapy. Further enhancement of PHA responsiveness was also demonstrated to nitrogen mustard and DNCB suggesting that such agents also may have adjunctive therapeutic value.

2. Two patients treated with immunotherapy alone had recurrences of their tumors before patients receiving chemotherapy or immunochemotherapy.

3. The majority of glioma patient's lymphocytes are less effective in killing their own tumor cells than are lymphocytes of normal controls, irrespective of which mode of therapy is given after surgery and radiation therapy.

4. Histologically, brain tumor biopsies from patients undergoing immunotherapy or immunochemotherapy, reveal lymphocytic infiltration of the leptomeninges and subpial areas, but no significant increase in numbers of lymphocytes within the substance of the brain tumors can be claimed because of the small amount of data and sampling errors due to biopsy limitations.

5. The animal model has shown that preimmunization and chemotherapy are effective means of treating the intracerebral murine tumor, that immunotherapy alone is a totally ineffective form of treatment, and that survival of animals treated with immunochemotherapy is not significantly, prolonged over that of animals treated with chemotherapy alone.

6. Computerized axial tomography can display the volumetric changes in tumor size after therapy but measurement errors due to the closeness of linear attenuation coefficients of tumor, necrotic tissue and edematous tissue remain a significant problem.

Significance of Bio-medical Research and the Program of the Institute:

1. As determined in a small series of patients and in experimental animals, immunotherapy of the specific typed tested alone is ineffective in treating brain tumors.

2. Intratumoral delayed hypersensitivity reactions to PPD alone are of questionable significance in the immunotherapy of brain tumors when no control of possible blocking factors or evidence of enhanced lymphocyte cytotoxicity is available.

3. Identification of the key factors as above will enable a more rational program of immunotherapy to be developed and tested.

Proposed Course:

1. A new prospective, randomized protocol comparing chemotherapy alone with immunochemotherapy will be developed. In this protocol the factor of radiotherapy and factors limiting immunologic effectiveness will be better controlled.

2. Cytotoxicity assays are being performed routinely on all brain tumor patients at intervals before and after entry into the trial.

3. Experiments are being undertaken to evaluate various cell treatments to determine which treatment stimulates maximal lymphocyte blastogenesis, and to correlate these *in vitro* findings with *in vivo* protection experiments.

4. The nature of "blocking factors" in glioma patients will be studied to determine the role of such factors (e.g. the "lymphocyte regulating globulin" of Horowitz and Brooks) in countering the effects of immunotherapy.

5. The tumor glycoproteins will be studies in relation to their tumor specificity and antigenicity (by immunological methods) as well as their chemical make-up by procedures such as lectin specificity, trypsinization, isolation and identification of the hydrolized surface products.

6. Develop and test a murine model of immunotherapy of gliomas where soluble tumor membrane extracts are the immunostimulant.

7. Evaluate the technique of computerized axial tomography as a method for following the volumetric and intrinsic structural changes in tumor size and composition after therapy.

8. Develop the sub-human primate model to further evaluate delayed hypersensitivity reactions in this species when more than one agent is used (e.g. PPD + DNCB given sequentially intracerebrally). The possibility of developing a larger animal model of glioma will also be investigated.

Honors and Awards: None

Publications:

Albright, L., Cohen, M.H. and Ommaya, A.K.: Intracerebral Delayed Hypersensitivity Reactions in Nonhuman Primates. Nature. (in press)

Ommaya, A.K. et al.: Combination Chemotherapy for Malignant Gliomas of the Brain. J. Neurosurg. (in press)

Ommaya, A.K. et al.: Computerized Axial Tomography: Estimation of Spatial and Density Resolution Capability. Br. J. Radiol. (in press)

Serial No. NDS(I)-63 SN/NR 1047(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Michael V. Green
Sybil J. Swann
Thomas H. Milhorat, M.D.
Archie W. Bleyer, M.D.

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radiosotope Section, Clinical Center, NIH

Department of Neurosurgery
Children's Hospital of D.C.

Pediatric Oncology Branch, NCI, NIH

Man Years:
Total: .4
Professional: .4
Others: .0

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospiral fluid itself will be obtained.

Methods Employed: The radioisotope cisternography and ventriculography procedures are now well established.

Recently we have devoted particular attention to one aspect of the CSF flow, i.e., its descent to the spinal subarachnoid space. Experiments have

been carried out in the Rhesus monkey after injection of radiopharmaceuticals within the ventricular system. The scintiphotographic data are appraised with the assistance of a computer. Digital analysis is performed using a small dedicated computer (HP 5407A Hewlett Packard Scintigraphic Data Analyzer), mated to the Anger camera. For this purpose the pin-hole collimator is positioned so that the entire lateral length of the cerebrospinal space is within the field of view of the Anger camera detector. Nine region of interest cursors are drawn: one over the cerebral convexity, and one to include the entire spinal subarachnoid space. Time-activity curves are obtained from each region of interest, simultaneously without moving the animal for the next three and one-half hours.

Preliminary experience has been gained in clinical material on the descent to the spinal subarachnoid space of the CSF, Eight patients, all newborn or infants,and all with abnormalities of the CSF circulation--the majority was made up of cases of myelomeningocele--have been subjected to the following procedure. A radiopharmaceutical has been injected into the lateral ventricles of the brain and the descent of the cerebral spinal fluid into the spinal canal has been studied with an Anger camera, and in selected cases with the help of a computer. When the computer has been used, total gathering of the data has been attained above the entire spine for a period of at least one hour following the intraventricular injection. A number of patients affected by meningeal leukemia, and in whom radioisotope ventriculography was carried out, were also followed up with spine scanning for the purpose of gaining experience with the spinal CSF descent.

Major Findings: During the current fiscal year we have:

1) Gained additional experience with cisternography in cases of porencephaly.

2) Added to the number of cases of supratentorial and hemispheric gliomas studied by radioisotope cisternography. The characteristic pattern of lack of ascent of the radiopharmaceutical on the tumoral side has been confirmed.

3) Injected radiopharmaceuticals within the lateral ventricles of Rhesus monkeys with the intent of studying the spinal CSF flow. A pattern of early spinal subarachnoid descent has been noted. This is followed first by equilibration, and later by decrease of spinal radioactivity as well as concomitant augmentation of cerebral convexity subarachnoid activity. When the radiopharmaceutical is introduced via the cisterna magna, the observed downward spinal pattern is even more marked, whereas an injection into the anterior basal cisterns is followed by a prevalent ascending direction of the tagged albumin toward the convexity of the brain.

4) Injected radiopharmaceuticals within the lateral ventricles of human patients (newborns, infants, and children) and followed the spinal descent of the radionuclide.

5) Gained additional experience with radionuclide ventriculography in patients with intracerebral neoplasm, meningeal infection and low pressure hydrocephalus. Important applications were assessment of CSF shunts, determining distribution of intrathecally administered drugs, evaluating functional status of hycrocephalus, and assessing the progression of neoplastic invasion of the ventricular system.

Significance of Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we now have a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoid pathways.

Last year's investigations should have practical implication for the diagnosis and follow-up of such conditions as porencephaly and hemispheric glioma.

The CSF spinal descent studies should enable us to determine what is the importance of the spinal CSF route of flow as an alternative pathway of resorption. The observations of the spinal descent pattern of the CSF have also heuristic significance in regard to a possible analysis of metabolites and drugs distribution through the CSF from the endocranial cavity to the spinal theca.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Honors and Awards: Dr. Di Chiro has been chosen for the annual 1974 Benedict Cassen Memorial Lecture at the Society of Nuclear Medicine in Los Angeles, California.

Publications: Di Chiro, G.: Radionuclide Studies of the 'Third Circulation.' Hospital Practice. 53-61, August 1973

Serial No. NDS(I)-65 SN/NR 1195(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radiographic and Radioisotopic Angiography of the Spinal Cord

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Louis Wener, M.D.
Jean R. L. Herdt, M.D.
Gerald S. Johnston, M.D.
A. Eric Jones, M.D.
Mark M. Mishkin, M.D.
Eugene L. Timins, M.D.

Cooperating Units: Department of Radiology, Cafritz Memorial Hospital, Washington, D.C.

Diagnostic Radiology Department, Clinical Center, NIH

Department of Nuclear Medicine, Clinical Center, NIH

Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

Medical Examiner's Office, City of Philadelphia, Department of Public Health, Philadelphia, Pennsylvania

Department of Neurosurgery, George Washington University Medical School, Washington, D.C.

Man Years:

Total:	.6
Professional:	.6
Others:	.0

Project Description:

Objectives: The introduction of cerebral angiography (1927) has markedly increased our knowledge of the vascular pathology of the brain. The vascular pathology of the spinal cord, on the other hand, remains a largely unexplored area.

Since 1964, we have been carrying out angiographic studies of the spinal cord and developed this technique into a reliable diagnostic tool. Selective injection of the contrast medium has made the difference between an occasional demonstration, and the consistent visualization of the spinal cord vasculature.

The usefulness of selective arteriography in cases of spinal cord arteriovenous malformations is now well established. We are continuing to use this technique to: 1) Learn more about the pathophysiology of the spinal cord arteriovenous malformations so that a better treatment of these important and frequent lesions may be developed. 2) Evaluate how useful spinal cord angiography is in cases of spinal cord tumors. 3) Establish whether or not this technique can be of diagnostic value in the study of obstructive spinal cord vascular disease. 4) Assess the usefulness of this technique in intervertebral disc pathology. 5) Evaluate the diagnostic possibilities of this procedure in posttraumatic spinal cord injury with or without vertebral fractures. 6) Establish the value and limits of newly introduced radioisotopic angiography of the spinal cord. 7) Explore possible emergency therapeutic means which could be employed to treat and cure, or at least minimize the effects, of the dreadful postangiographic cord complications. 8) Acquire new information regarding the fine vasculature of the human spinal cord, with particular emphasis on the intrinsic vessels (sulcal or central arteries and other perforating or penetrating branches). This goal is accomplished by postmortem microangiographic techniques in cadavers of all age groups. We are paying particular attention to cords of aged adults.

Methods Employed: Selective arteriograms with modern catheter techniques are carried out in patients in whom spinal cord vascular or tumoral lesions are suspected. The subtraction technique is used to better visualize the injected vessels. In addition, in the last fiscal year we have gained considerable experience with the direct radiographic magnification angiograms.

For the technique of radioisotope angiography of the spinal cord a bolus of 15 mCi of 99mTc human serum albumin (1 to 2 ml) is injected in a left antecubital vein. Immediately afterwards, cinescintiphotographic or rapid flow Polaroid views of the various segments of the spine are obtained with an Anger scintillation camera. In the last fiscal year our scintiphotographic data have been significantly ameliorated by a computer assisted analysis and reconstruction of images, as well as by isometric contour computer display of the data.

For the postmortem studies of the vessels of the human spinal cords, (aged adults) we have used our previously developed microangiographic techniques.

Based on the observation, made elsewhere, that in two patients who died soon after aortography with spinal cord complications, the iodine content in the CSF was enormously increased, we are attempting an emergency therapeutic method consisting of flushing out the "iodine contaminated" CSF.

Major Findings: During the current fiscal year we have been able to further develop the technique and to augment our experience with selective arteriography of the spinal cord. In particular:

1) Additional experience has been gathered with arteriovenous malformations and hemangioblastomas of the spinal cord.

2) We have reviewed our entire material of gliomas of the spinal cord. Myxopapillary ependymomas of the conus and filum terminale present characteristic--pathognomonic?--angiographic findings: enlarged spinal arteries, striking visualization of the anastomotic loop(s), markedly slow circulation.

3) We have increased our experience with obstructive vascular disease of the spinal cord.

4) Additional cases of arteriographic studies of the spinal cord in patients with posttraumatic para- and tetra-plegia have been carried out. Direct radiographic magnification has been particularly useful in these cases. The most impressive finding in these cases has been the discrepancy between the gross bony-ligamentous damage and the relative sparing of the vascular system. It is remarkable that in cases of total posttraumatic tetra- or para-plegia and massive skeletal-ligamentous injuries, the continuity of the vascular pathways (at least the large vascular channels) may be intact.

Three "firsts" have been attained in our angiographic studies of spinal cord injuries:

I) We have demonstrated, by high magnification, the sulco-commissural arteries in one patient.

II) Extravasation of contrast medium into the epidural space has been shown in one case, indicating "ongoing" bleeding.

III) One of the tetraplegic patients whom we had studied by angiography, died several weeks later. We succeeded in obtaining a microangiographic cord study. In this instance in vita angiography and postmortem microangiography of the cord may be compared.

5) We have studied many patients with spinal cord pathology due to intervertebral disc disease. Particularly rewarding has been the information obtained in cases of herniation of the thoracic discs (four patients). Direct radiographic magnification has been expecially useful in these cases. Only by spinal cord arteriography, we can reach a definite diagnosis of this condition together with an evaluation of the degree of impingement on the cord and its vessels by the protruding disc. This diagnosis is indispensable for a correct surgical therapy of this pathological condition: The exposure of the herniated thoracic disc should not be achieved through posterior laminectomy, but rather through a lateral retropleural or lateral transpleural approach. Our experience with angiography in cervical spondylotic myelopathy is now large (over 20

cases). We are evaluating this material.

6) We have continued our exploration of the angiographic findings in cases of suspected postradiation myelitis of the spinal cord.

7) A large number of patients have been studied with radioisotope angiography of the spinal cord. Striking demonstration of such lesions as arteriovenous malformations and hemangioblastomas are obtained by this simple, innocuous, and informative method. No diagnostic technique can match radioisotope angiography of the spinal cord in the screening and follow-up of patients with progressive myelopathy and suspected of harboring arteriovenous malformations or vascular tumors of the spinal cord.

8) The microangiographic evaluation of the aged human cord and a comparative appraisal of the intrinsic vasculature in the various segments (cervical, high- and mid-thoracic,and thoracolumbar) of the cord is underway.

9) In several patients who, in other hospital centers, developed paraplegia immediately after abdominal aortography, flushing out of the CSF and substitution with normal saline solution has been carried out, on an emergency basis, under our suggestion and guidance. This lavage has resulted in every instance in a rapid amelioration of the paraplegia. The improvement has been concomitant with a decrease of the CSF iodine content. For comparison purposes we have been gathering CSF samples during the various stages of different angiographic procedures, particularly selective arteriography of the spinal cord. In patients without neurological complications the postangiographic raise of the iodine content in the CSF in minimal.

Significance to Bio Medical Research and the Program of the Institute: Radiographic and radioisotopic angiography of the spinal cord are increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: We intend to concentrate our efforts on the angiographic evaluation of posttraumatic tetra- and para-plegia. Considering that by selective techniques we may bring the injectant directly into the damaged area, it is conceivable that we could introduce in this district drugs antagonists to the pressor amines. (These amines are apparently released and contribute to the spreading of damage in the traumatized cord (Osterholm)). Thus, a diagnostic technique could be extended to be also a therapeutic method.

We are "watching" for possible further technical developments of the technique of selective arteriography of the spinal cord. We have recently established the value of direct radiographic magnification, and we are considering initiating the use of angiotomography for a better visualization of the smaller vessels, possibly the intrinsic arteries and veins of the cord.

Improved X-ray vascular contrast media will also enhance the diagnostic possibilities of spinal cord angiography. We are following, very closely, the

recent developments in the area of polimeric, ion-balanced and non-ionic iodinated X-rays contrast media.

We will dedicate much of our attention to technical improvements in the newly introduced radioisotope angiography of the spinal cord. This method, which we are extensively using as a screening and follow-up procedure,could become a more definitive and informative diagnostic examination. By increasing our resolution through a computer-assisted reconstruction and enhancement of the images, we should be able to extract a lot of diagnostic information from this simple and innocuous technique.

Postmortem microangiography of the aged adults' cords should offer new insights on such conditions as obstructive vascular disease of the cord due to arteriosclerosis and cervical apondylosis, and possibly on degenerative and demyelinating cord diseases.

Honors and Awards: None

Publications:

Di Chiro, G., Jones, A. E., Johnston, G. S., Allen, F. H.: Value and Limits of Radionuclide Angiography of the Spinal Cord. RADIOLOGY 109:125-130, October 1973

Di Chiro, G., Wener, L.: Angiography of Ependymomas of the Spinal Cord and Filum Terminale. Amer. J. Roentgenol. (In Press)

Di Chiro, G., Wener, L.: Radiographic and Radioisotopic Angiography of AMV and Hemangioblastomas of Spinal Cord: Proceedings of Fifth International Congress of Neurosurgery - Tokyo. Excerpta Medica (In Press)

Wener, L., Di Chiro, G., Gargour, G. W.: Angiography of Cervical Cord Injuries. RADIOLOGY (In Press)

Serial No. NDS(I)-66 SN/OC 1245(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

Previous Serial Number: Same

Principal Investigator: Paul Fedio, Ph.D. and William Sheriff, M.A.

Other Investigators: J. Van Buren, M.D., Ph.D.; A. Ommaya, F.R.C.S.; Monte Buchsbaum, M.D.

Cooperating Units: Section on Technical Development, NIMH; Adult Psychiatry Branch, NIMH

Man Years:

Total:	0.4
Professional:	0.3
Other	0.1

Project Description:

Objectives: To identify brain mechanisms in man which mediate perception, and the storage and retrieval of information; to evaluate the significance of brain dysfunction in neuropsychiatric disorders.

Methods Employed: Auditory and visual perceptual tests were used, the stimuli being patterned to yield either meaningful or nonsense information. Electroencephalographic (EEG) activity was recorded from scalp electrodes positioned over the left and right hemispheres. Included for study were patients who had undergone unilateral temporal lobe resections for the relief of intractable epilepsy, patients with chronic, indwelling electrodes implanted in the thalamus or temporal lobe, and patients with psychiatric disorders in the absence of obvious neurologic problems.

Major Findings: All electrographic test runs were conducted off-line and the evoked potential data for cognitive parameters is currently being processed. The study involving neuropsychiatric patients is in progress.

Significance to Biomedical Research and the Program of the Institute: Behavioral data available from epileptic patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits which are

related to the laterality of temporal surgery and to the specific character of the material. The technique employed in this project affords a more precise method for outlining the network of cortical and subcortical structures in the human brain which support learning and memory. The research also provides direct comparisons of patients with neurologic or psychiatric disorders in order to identify possible brain dysfunctioning in the thought or affective disorders in schizophrenia or psychosis.

Proposed Course of the Project: To develop additional tasks and program for EEG data analysis with wider application to patients with different neurologic or psychiatric disorders.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 SN/NR 1413(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Floro Miraldi, M.D., Ph.D.
Eugene T. Yon, Ph.D.

Cooperating Units: Department of Biomedical Engineering, and University Hospital, Case Western Reserve University, Cleveland, Ohio

Man Years:

Total:	.1
Professional:	.1
Others:	.0

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if an effective and efficient method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintigraphic record of the head would be attained by tomography. We have been theoretically analyzing the various methods tried by other investigators to solve the problem of scanning tomography. These methods fall into four general categories: 1) focusing collimator systems; 2) systems using

collimators inclined to the plane of interest; 3) scanning with collimators in the plane of interest; 4) time-of-flight positron systems.

We have performed a mathematic appraisal of the three fundamental parameters: Sensitivity, resolution, and efficiency.

We have proposed a new approach and built a prototype device: The strong focusing multi-layered Tomoscanner.

Major Findings: The prototype Tomoscanner is in Cleveland, where significant modifications have been recently carried out. In particular, the angulation of the detecting probe has been changed to 30 deg, which seems to represent a satisfactory compromise.

Preliminary experience with patients using the improved Tomoscanner has proven promising. About 30 cases suspected of harboring intracranial space occupying lesions and a few patients with liver pathology have been studied so far. The blurring of the activity outside the plane of interest is clearly accomplished. By multiple sequential cuts of the head a layer by layer analysis is possible.

For a definite assessment of the true capability of the Tomoscanner a large number of patients must be studied.

Significance to Bio-Medical Research and the Program of the Institute: If the resolution of brain scanning can be significantly improved by tomography, cerebral scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by other diagnostic neuroradiologic methods, would then probably be "picked up." An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods, taken as a "whole" would then occur.

Proposed Course of Project: To attain the goal of studying a large number of neurologic and other patients by tomoscanning a simple playback system of the tape on which the data are digitalized and stored is indispensable. An indexer (locator system) has been built and this will allow us to perform a rapid reanalysis of the information accumulated on the tape. With the locator we will be able to proceed in a rapid fashion with the appraisal of the equipment's capabilities.

Particular attention will be devoted to the evaluation of "Tomoscanning" in clinical material in conjunction with conventional brain scanning, especially in patients with brain tumors and other endocranial space occupying lesions. The Tomoscanner will also be evaluated in association with the techniques of radioisotope cisternography and radioisotope ventriculography. A limited amount of work with tomographic scanning in experimental models (monkeys which have been subjected to experimental stroke and monkeys in which

radioisotope ventriculography is performed) is also anticipated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 SN/OC 1417(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Joseph Fenstermacher, Ph.D., Barbro Johansson, M.D.

Cooperating Units: C-LCP

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objective: To determine the resistivity of nervous tissues under various neurosurgical conditions.

Methods Employed: Cats under Fluothane anesthesia, hypothermia, hypertension, etc. were used. In one experiment, a monkey was used who was subjected to electrical measurements for a period of two months. The method of measuring electrical resistance of the tissue was previously described (Li, et al. 1968). With this method, the results were applied to the Maxwell's Equation and the extracellular space of the tissue was calculated. The calculated extracellular space was then compared with the findings obtained by the concentration profiles of radioactively labeled compounds following subarachnoid perfusion. The brains of the animal after the experiment were fixed, sectioned and stained with Luxol Fast Blue-Nissl and Luxol Fast Blue-PAS stains for histological examination.

Major Findings: The extracellular space of the cat's cerebral cortex was found to be between 15 and 20% and was unchanged when the temperature of the cortex was decreased from 37 to 15° C. The rate of CSF formation was, however, found to be markedly reduced by hypothermia.

In another series of experiments, a sudden increase of systemic blood pressure by 90 mmHg was found to break down the blood brain barrier. With the blood brain barrier broken down, the activity of the nerve cells was found to be changed or inhibited.

The results from experiments on brain edema show changes in the level of consciousness which are still being subjected to analysis.

Significance to Biomedical Research and the Program of the Institute: Change in extracellular space may be a measure of extracellular or intracellular edema of the cells. The latter, in turn, is a measure of the metabolic response of the nerve cells to various pathological or physiological conditions. It is hoped that the method herewith described will be applied to neurosurgical patients as a measure of the progress or improvement of the neurological conditions.

Proposed Course of the Project: Further study along this line will be made when more supportive research personnel are available and when the previous collected data are published.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 SN/OC 1424(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions

Previous Serial Number: Same

Principal Investigators: Paul Fedio, Ph.D. and Ayub K. Ommaya, F.R.C.S.

Other Investigators: J. Van Buren, M.D., Ph.D.; T. Zahn, Ph.D. and W. Sheriff, M.A.

Cooperating Units: Laboratory of Psychology, NIMH and Section on Technical Development, NIMH

Man Years:

Total:	0.2
Professional:	0.1
Other	0.1

Project Description:

Objectives: To identify structural-functional interplay of limbic brain mechanisms which regulate emotional and intellectual behavior in man.

Methods Employed: Therapeutic benefits from bilateral cingulomotomy in reducing "appreciation of pain" are examined by psychophysiologic procedures before and after surgery. In terms of cognitive behavior, perception and immediate memory were monitored during electrical stimulation of chronic electrodes implanted in the cingulum and other structures in the limbic system.

Major Findings: No patients were added to this research project during the past year. Computer programs are currently being developed to: 1) evaluate the effects of surgery in changing the patients' tolerance of pain in terms of psychophysiological responsivity, 2) evaluate the effects of limbic lesions on personality and emotional status.

Significance to Biomedical Research and the Program of the Institute: This project provides an opportunity to define precise behavioral and autonomic concomitants of limbic functions in man. The observations contribute to

a better understanding of neuropsychologic mechanisms involved in the emotional appreciation of learning and memory, pain and stress.

Proposed Course of the Project: To continue the study and to extend the protocol to include patients subject to therapeutic lesions in the temporal lobe.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1526(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objective: To study the electrical properties of the individual nerve cells in the cerebral cortex under normal and induced pathological conditions.

Methods Employed: Glass micropipette electrodes were inserted into the cells in the motor cortex of anesthetized cats. The electrical activity of the cells were recorded; and, thereafter, electrical current of increasing intensity was applied. The electrical resistance of the cell was derived from the current-voltage ratio and the time constant of the cell membrane and capacitance were calculated. This was carried out before and after the application of strychnine to the exposed cortex.

In another series of experiments repetitive electrical stimulation was applied to the cortex through a gross electrode adjacent to the recording micropipette electrode, or to the individual cells through the recording micropipette electrode.

Major Findings: Some of the electrical properties of cortical neurons have been reported previously. The preliminary observations suggest that they are different from neurons under the effect of strychnine. For instance, the resistivity of normal cortical neurons measures $(9.17 \pm 2.68) \times 10^6$ ohms while that of the epileptic neuron was found to be in the order of 5 to 6 $\times 10^6$ ohms. Further, epileptiform activity of the cortex requires "synchronous" presynaptic bombardments and there is good evidence of negative feedback through the axon collaterals to the epileptic neurons.

Significance to Biomedical Research and the Program of the Institute: The electrical properties of the cell determine the cell's "natural" behavior as well as its response to the environment. If the electrical properties of the epileptic neurons are different it seems likely that any effective treatment will restore the normal electrical properties. Similarly, the electrical measurements may serve as a criterion of the effectiveness of the treatment of prognosis of the epileptic disorder.

Proposed Course of Project: Further investigations will be carried out to determine the electrical properties of nerve cells in response to various convulsants and anticonvulsants.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1527(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathways.

Methods Employed: Intracellular and extracellular micropipette electrodes were used to record the activity of the nerve cells in the motor cortex of the cat and stimulation was applied to the basal nuclear structures. In the past year stimulation was applied primarily to the caudate nucleus and nucleus ventralis lateralis of the thalamus.

Major Findings: The discharges of cells in the motor cortex increase or decrease and the membrane potential increases or decreases in response to stimulation dependent upon the pre-stimulation activity of the cell and the duration of stimulation. The results of this experiment are in the process of analysis for publication.

Significance to Biomedical Research and the Program of the Institute: This study provides further information about the interaction of the pyramidal and extrapyramidal system at the central level. The present investigator is aware, however, that the regulatory effect of the extrapyramidal system on motor function may also be found in the cells of the motor cortex itself because only 4-5% of the cells in the motor cortex have descending axons in the medullary pyramid. The accumulated data may eventually shed some light on the understanding of motor function of dysfunction.

Proposed Course of Project: Experiments have been planned on unanesthetized monkeys for the further study of the motor system. These experiments will employ multiple microelectrodes to record simultaneously single cell activity from various nervous structures. The activity of the cells will be co-related with the movement of the monkey. Thereafter, electrolytic lesions in certain basal nuclei will be made and clinical evaluation and electrophysiological studies will be repeated. Therapeutic agents such as L-dopa, reserpine, etc. will then be given and similar clinical and physiological studies will be carried out.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/NR 1654(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Spinal Cord Angiography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Mary K. Hammock, M.D.
Jean R. L. Herdt, M.D.
Jack Fein, M.D.
Giulio J. D'Angio, M.D.
Kenneth Earle, M.D.
Eugene L. Timins, M.D.

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Armed Forces Radiobiology Research Institute, Bethesda, Maryland

Department of Radiation Therapy, Memorial Sloan-Kettering Cancer Center, New York, New York

Armed Forces Institute of Pathology, Washington, D.C.

Department of Neurosurgery, George Washington University Medical School, Washington, D.C.

Man Years:

Total	.2
Professional:	.2
Others:	.0

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established.

In order to expand the clinical applications of arteriography of the spinal cord we are working with experimental angiographic and microangiographic models in primates.

Previously, we have concentrated our attention on the area of experimental obstructive vascular disease of the spinal cord in the Rhesus monkey. In the past fiscal year much of our experimental investigation has dealt with an iatrogenic pathological condition: postradiation myelomalacia (myelitis).

In the area of postradiation myelitis we are particularly interested in establishing whether the basic pathological lesion of this dreadful complication is primarily neurogenic or vascular.

Methods Employed: Preradiation angiographic studies (selective technique) of the thoracolumbar segment of the spinal cord are carried out in young, healthy Rhesus monkeys. Soon after, selective irradiation of the thoracolumbar cord using the LINAC accelerator (A.F.R.R.I.) is initiated. Total dosage and modalities of delivery are chosen to approximate the radiation protocol which most often seems to cause myelomalacia in human patients.

At the end of the radiation the monkeys are kept under careful observation for periods of many months. Neurological testing of the lower limbs is performed twice a week. If and when the monkeys show signs of developing or established paraplegia, repeat selective arteriography of the irradiated segment is carried out. Following this, the animals are perfused for microangiography of the spinal cord and then sacrificed. The cord is studied by gross observation, microangiography, routine histology and special myelin stains. Careful gross and histological analysis of the neighboring aortic segment, its branches and the pertinent radiculomedullary arteries is also carried out.

Major Findings: During the past fiscal year three irradiated monkeys have developed postradiation myelitis. This condition occurred six to eight months after the end of irradiation. We are evaluating the angiographic, microangiographic and histological data obtained. We have lost a large number of animals due to nonneurologic (most frequently hemorrhagic enteritis) postradiation damage.

Significance to Bio-Medical Research and the Program of the Institute: We should be able to shed some light on the pathogenesis of the postradiation myelitis. This is not a rare complication in human patients (over 500 cases have been reported in the literature).

Proposed Course of Project: Appraisal of the postradiation data which we have already collected as well as new data in other irradiated animals now under observation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1658 (c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Hemispheric Development and Specialization of Intellectual Functions

Previous Serial Number: Same

Principal Investigators: Paul Fedio, Ph.D. and John Van Buren, M.D., Ph.D.

Other Investigators: A. Ommaya, F.R.C.S.; M. Mishkin, Ph.D.; N. Butters, Ph.D.

Cooperating Units: Laboratory of Psychology, NIMH; Veterans Administration Hospital, Boston, Massachusetts

Man Years:

Total:	0.8
Professional:	0.6
Other	0.2

Project Description:

Objectives:

1. Outline brain mechanisms which support speech and language functions, and code information to be held for immediate use (short-term) or for later, delayed recall (long-term).

2. Investigate whether the human brain registers visual and auditory information in different cortical areas, and whether the verbal or nonverbal character of the information is vital to this neural process.

3. Compare the effects of brain lesions during infancy vs. adulthood the development and recovery of intellectual abilities.

Methods Employed:

1. The laterality and general outline of cortical zones instrumental in perception and immediate memory of verbal and nonverbal material were mapped by stimulation of the cortex during neurosurgical treatment of epileptic patients. The behavioral tests utilized verbal or nonverbal materials: a series of photographs of common objects and random visual patterns. In each e, the subject was instructed to name the object or to discriminate

several patterns: a short term memory command was also included for each task.

2. The organization of cortical and subcortical systems which enable man to speak and to use language or to remember events over varying periods of time was studied during electrical stimulation of therapeutic electrodes in thalamic nuclei. Behavioral indices during stimulation were monitored on the object-naming task and a random-form discrimination test described earlier.

3. A profile of perceptual and intellectual deficits was plotted for neurosurgical patients with focal damage limited to the parietal or temporal lobes. The test protocol includes several visual and auditory memory tasks which study the effects of distraction or interference on learning. In a separate project, imagery, or the "ability to see something in the mind's eye," is being studied in patients with injury to the left or right temporal lobe.

Major Findings:

1. The ability to identify objects and to remember their names was studied during electrical stimulation of the exposed surface of the brain in patients undergoing surgery for the relief of epilepsy. This speech mapping procedure identifies by electrical stimulation those cortical areas of the brain that are essential for the preservation of language.

The general findings conform to established observations that language and related verbal processes rely upon an intact left brain. In the posterior temporo-parietal regions of the left hemisphere, we were able to map distinct areas which support the ability to identify pictorial verbal material. Stimulation of the anterior cortical areas of the left temporal lobe and a wide area of the right brain failed to disrupt naming skills.

There appears to be a functional hierarchy within the left (and probably the right) hemisphere with memory as a common process. With severe interference or insult to the left posterior system, the basic machinery of naming simple objects was disabled. In minor form, the basic processes remain intact, and the impairment reveals itself as a loss in immediate memory. Electrical stimulation adds noise to the system so that older traces of verbal material (names of familiar objects) are not as likely to be disrupted as the younger trace of a stimulus recently seen and registered.

Memory presses the anterior and posterior temporal regions into different support services. The primary or immediate memory system may be intimately linked with the anterior temporal lobe and medial structures (hippocampal complex), while the posterior temporo-parietal cortex may support secondary or long-term memory. Our findings correlate anterograde memory errors with stimulation of the anterior temporal lobe, and retrograde errors with posterior temporal stimulation. With anterior cortical stimulation, information could

be retrieved from an immediate memory store, but newly perceived information could not be deposited into the same storage system for subsequent recall. This may be a defect in the consolidation rather than retrieval, mechanism in that stimulation prevents the memory trace from being established for immediate recall.

In contrast, with cortical stimulation of the posterior temporal region, recently stored information could not be retrieved even though other information could be simultaneously transferred into the same storage system. This indicates that, in man, memory for recent and remote information may be served by a common retrieval mechanism. Therefore, patients who suffer stroke or other forms of insult which invade the primary language zones are apt to display aphasia and a significant memory disorder.

2. Mechanisms for perception and memory were probed by electrical stimulation via therapeutic electrodes in the lateral thalamus. Stimulation within the left pulvinar nucleus induced transient dysphasia and a retrograde loss in recent memory for verbal memoranda. In contrast, comparable stimulation of the right pulvinar failed to disrupt verbal behavior and instead, interfered with perception and recognition of complex visual patterns. The findings suggest that an asymmetry in the functional organization of linguistic and nonverbal processes appears to exist at the level of the lateral thalamus.

At first glance, the left and right pulvinar nuclei behave like the ipsilateral cortex with regard to identifying, registering and retrieving from immediate memory, verbal and nonverbal material.

However, the behavioral deficits accompanying cortical and subcortical stimulation appear to be different. Specifically, the data suggest that dysphasia and amnesia were inseparable during cortical, but not subcortical, thalamic stimulation. That is, if the patients were unable to name objects during cortical stimulation, they also experienced severe memory impairment. With thalamic stimulation, recent memory was occasionally spared while at the same time, the patient experienced dysphasia. This may suggest that the registers or stores that are vital for coding information are dependent upon cortical mechanisms. The contribution of the pulvinar, and other posterior thalamic nuclei may be limited to pre-perceptual processing, that is, these nuclei attenuate and shuttle sensory information to the cortical stations for interpretation and analysis.

A separate study involved stimulation in the more anterior thalamic nuclei and adjacent strio-capsular structures. The behavioral observation, in brief, indicate that these structures support speech by providing attentional or orientation cues for incoming stimuli [See report by Dr. John Van Buren, Serial No. NDS(I)-55 SN/OC 200(c)].

3. In collaboration with Dr. Butters, Aphasia Unit, Boston VA Hospital, patients with parietal lesions or Korsakoff's Syndrome (VA Hospital), or with temporal lobe lesions (NINDS) are being studied on a wide range of memory tests.

Preliminary data show that Korsakoff patients (with presumed subcortical or limbic damage) are more susceptible to the effects of distraction during learning than patients with cortical injury regardless of the type of material to be memorized. The early data returns also suggest that cortical lesions in the left and right hemisphere have a differential effect on learning visual (right) and auditory (left) information.

4. In a study with Dr. Mishkin, Laboratory of Psychology, NIMH, the perceptual deficits that accompany temporal lobe injury were partitioned according to an imagery factor. On the basis of preliminary results, it appears that patients with right temporal damage have trouble forming visual images and are unable to use visual images as mnemonic cues to offset learning disabilities. In contrast, patients with left temporal injury, may be able to form in their "mind's eye" a picture of an object which they may be unable to name due to aphasia, and to use this technique to compensate for learning difficulties.

Significance to Biomedical Research and the Program of the Institute: The investigations advance understanding of the development and organization of structural-functional relationships in the human central nervous system. The information also attends to the impact of cerebral dysfunctioning in producing dysphasia, dyslexia and kindred language disorders.

Proposed Course of the Project: The visual and auditory tasks will be modified to further delineate immediate and long-term memory impairment in patients with lateralized cortical and subcortical lesions. Parallel studies of interhemispheric relations will be made during deep brain stimulation and during cortical stimulation of patients in the neurosurgical operating suite.

Honors and Awards: None

Publications:

Fedio, P. and Van Buren, J. M.: Memory deficits during electrical stimulation of the speech cortex in conscious man. Brain and Language: 1, 29-42, 1974.

Fedio, P. and Van Buren, J. M.: Memory and perceptual deficits during electrical stimulation in the left and right thalamus and parietal subcortex. Brain and Language, (in press).

Serial No. NDS(I)-69 SN/NR 1791(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Angiography of Cerebral Venous Obstructive Disease

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: Kenneth Earle, M.D.

Cooperating Units: Experimental Surgery and Clinical Medicine Section, Veterinary Resources Branch, Division of Research Services, NIH

Armed Forces Institute of Pathology, Washington, D.C.

Man Years:
Total: .0
Professional: .0
Others: .0

Project Description:

Objectives: "Stroke" is one of the pathological conditions which recently has been a target attacked from many different sides. For instance, in the last ten years, a great amount of research has been dedicated to the angiographic evaluation of the cerebrovascular obstructive disease. The major emphasis, however, has been put on the arteriographic findings. A perusal of the literature clearly shows that only scattered observations have been made regarding the phlebographic changes. The neuropathological studies (Hahn, Loeb, Zülch), on the other hand, have pointed out the fact that the obstructive venous pathology, although not as frequent as its arterial counterpart, represents an important entity.

We intend to explore the angiographic changes occurring in the cerebral vessels after experimental obstruction of the various brain venous channels (veins and dural sinuses) in a primate experimental model. We are particularly interested in establishing, on the basis of these experiments, which venous occlusions can be well tolerated and which are, instead, incompatible with good function. We will try to appraise, again angiographically, what types of compensatory mechanisms take place after the venous ligations. Do new venous draining pathways open, and if so, which and how efficient are

these reserve discharge routes? The angiographic work will be carried out in close connection with an analysis of the clinical status of the operated animals.

The knowledge acquired in our experimental models will be extended to human material.

Methods Employed: Catheter arteriographic studies in the monkey (Macaca mulatta) are performed before and after ligation of one or several of the large endocranial venous channels.

Together with the post surgical angiographic evaluation, an analysis of the clinical condition of the monkeys is carried out. After various periods of time following the venous ligations, the animals are finally sacrificed in order to carry out microangiographic and gross and microscopic neuropathologic studies.

We are planning microangiographic studies of brains (supplied by the AFIP)of patients who have died of possible cerebral thrombophlebitis.

Major Findings: We have acquired a significant degree of experience in the evaluation of the monkey cerebral angiography. We have been impressed by the remarkable similarity of the major, and even medium size vessels (arteries, veins, dural sinuses) in the Rhesus monkey and man. We have good evidence, therefore, that the primate model will be applicable to the goals of our research project.

We have carried out in a group of Rhesus monkeys, ligation of the superior longitudinal sinus in its anterior, middle or posterior third. Cerebral angiographic studies have been performed before and at various time intervals after the ligation. We are now in the process of evaluating our angiographic, surgical, gross and light microscopic anatomo-pathologic findings in the operated monkeys.

Significance to Bio-Medical Research and the Program of the Institute: This project can increase our understanding of the pathophysiology of the cerebrovascular occlusive disease. Possibly, we will be able to recognize and separate specific conditions (mainly due to venous drainage impairment) in the large group of pathologic entities which are classified under the heading of "stroke."

Proposed Course of Project: We intend to continue our experimental investigation in monkeys.

Later on we intend to apply the acquired experimental knowledge to the human clinical experience.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 SN/NR 1866(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Cerebral Blood Flow in Normal and Infarcted Brain by Radiographic and Radioisotopic Methods

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro, M.D.
Mary K. Hammock, M.D.

Other Investigators: Harry Agress, Jr., M.D.
Michael Green
Gerald D. Johnston, M.D.
A. Eric Jones, M.D.
Sybil J. Swann
George S. Flinn, M.D.
Kenneth Earle, M.D.

Cooperating Units: Laboratory of Applied Studies, Division of Computer Research and Technology, NIH

Department of Nuclear Medicine, Clinical Center, NIH

Diagnostic Radiology Department, Clinical Center, NIH

Armed Forces Institute of Pathology, Washington, D.C.

Man Years:

Total:	.6
Professional:	.6
Others:	.0

Project Description:

Objectives: Although an enormous number of studies have been carried out on the cerebral blood flow (CBF), the actual behavior of the small cerebral vessels (arteries, capillaries, and veins) in physiologic and pathologic conditions is not yet clear. In fact, in recent years, new concepts--luxury perfusion, intracerebral steal, blue arteries and red veins, loss of autoregulation--have been introduced. These concepts have not brought about increased clarification, but rather additional confusion. We are convinced that morphological analysis of the vessels still remains a potential source of significant information in the area of normal and pathological CBF. We

intend, therefore, to pursue the matter of extracting information regarding the circulatory conditions of the brain from radiographic observations, substantiated in selected instances by radioisotopic methods and possibly other diagnostic and research techniques.

Methods Employed: Radiographic and radioisotopic observations of the cerebral circulation represent the main tools to be used in this study.

An experimental stroke model in Rhesus monkeys has been developed. The middle cerebral artery is occluded at its origin through the superior orbital fissure after the removal of the eyeball. This technique(Garcia) offers various advantages. Particularly important is the fact that the produced infarction is consistent in its anatomical location (medial-inferior district of the ipsilateral basal ganglia) and its extent. Also, because no craniectomy is needed, subsequent studies with radioisotope scanning are easily interpretable.

After production of the anemic infarction our multi-pronged evaluation includes:

A) Periodic neurologic testing.

B) Periodic conventional static radionuclide brain scanning.

C) Periodic magnification internal carotid angiography (an indwelling catheter is positioned in the internal carotid artery of some of the stroked monkeys).

D) Periodic radioisotopic angiographic studies (Tc pertechnetate) and periodic ^{133}Xe washout studies (through indwelling internal carotid catheter).

All the radionuclide flow data are gathered with an Anger camera and stored on tape. Subsequently, the data on tape are analyzed by a computer-assisted program. Multiple cursors are drawn in the central part and at the periphery of the infarcted area. The purpose of this approach is to evaluate the centripital and centrifugal blood flow in the infarcted area at various stages of development of the infarction. The data should be of assistance in the evaluation of the circulation parameters in the initiating, evolving, complete, and resolving stroke. The centripital and centrifugal flow is evaluated second by second after the intracarotid injection of the radiopharmaceutical.

E) Acute polarographic deep electrodes are used in some of these animals for recording oxygen availability.

F) At various time intervals after the creation of the stroke, the animals are sacrificed by a "sudden death" technique and immediately afterwards perfused with a special micropaque solution for postmortem microangiographic studies.

G) The "sudden death" is accomplished under various attempts at modifying the pathophysiology of the CSF: hyperventilation, hypercarbia, anoxia, hyper- and hypo-tension, use of vasodilators.

H) In a few monkeys perfusion with the radioiodinated albumin macro-aggregates for scanning appraisal of the infarcted hemisphere has also been carried out.

I) In selected monkeys the changes of the superficial vasculature are followed up by means of fluorescein angiography of the exposed cortical surface.

J) A day-by-day histological analysis of the anemic infarction is under way. Stroked monkeys are sacrificed for this purpose, one at each sequential day, after the production of the stroke.

K) We plan to study the infarcted monkeys by computerized axial tomography (C.A.T.) as soon as a device for this technique (EMI-Scanner, ACTA-Scanner) will be available to us.

L) We are getting ready to start "revascularization" of our infarcted monkeys with an original method of by-pass procedure to the middle cerebral artery distal to the occlusion. The "revascularized" monkeys will be subjected to all the above multi-pronged testing.

Major Findings: We are very pleased with the experimental stroke model which we have developed. The possibilities of investigation with this model are practically unlimited. We have started evaluating the many data available to us from the first group of infarcted monkeys.

1) The stroke model used gives a consistent area of infarction in deep cerebral layers with a minimal amount of brain exposure.

2) Microangiograms prepared in the manner described can fix at a point in time the dynamic changes taking place in the microvasculature.

3) The method allows us to duplicate in deeper structures some of the observations of others on the reaction of surface vessels.

4) We can demonstrate changes in vessel morphology following $PaCO_2$ changes both in normal and infarcted monkeys.

5) Both hypo and hypercapnia decrease the perfusion of zones of ischemia during the acute phase. Hypocapnia accelerates the rate of ischemia to a greater extent than hypercapnia.

6) In completed stroke at the five day interval, hyperventilation hypocapnia may increase perfusion of nonischemic portions of the brain as compared to normocapnic and hypercapnic animals.

7) By polarographic depth electrodes determination a reversal of the normal O2a response in the ischemic brain to either low or high $PaCO_2$ levels has been observed. This would suggest that attempts at manipulation of cerebral blood flow to ischemic or infarcted brain using hypo or hypercapnia would not be consistently successful due to the variable response obtained.

8) A correlative study between sequential radionuclide brain scanning and time-lapse microangiograms has been completed. In the majority of animals brain scintigraphy becomespositive by two weeks to regress toward negative by four to six weeks after the ligation of the middle cerebral artery. The increased radioisotope uptake in the affected area is clearly related to neovascularization around the area of infarct as shown by the microangiograms. Decreased vascularity, peripheral gliosis and central cavity formation are the main factors determining the diminution of the radionuclide penetration in the involved area at later stages.

Significance to Bio-Medical Research and the Program of the Institute: We are hopeful that the computer analysis of our radioisotope angiography data in and around the infarcted area will be informative. In particular the determination of the centripetal and centrifugal blood flow in the area of the lesion should enable us to draw some conclusion on the mechanisms of resolution of the infarction and perhaps a better understanding of the important phenomenon of the luxury perfusion.

We are confident that with our stroke model we will be able to contribute both in the basic area of CSF pathophysiology of the infarcted brain, as well as in some practical management of the patients with stroke. Perhaps we will shed some light on such fundamental questions as: a) if and when we should hyperventilate patients with stroke; b) when, if ever, induced hypercarbia may be useful in these patients; c) what is the optimal level at which the blood pressure should be maintained during the various stages of the stroke; d) if, when, and which vasodilators should be used.

We have hopes that our "revascularization" approach may prove to be efficacious, practical, and applicable to human patients.

Proposed Course of Project: This is a long-range research project. The evaluation of the cerebral blood flow both in experimental models and in clinical material is still in its infancy, but we are convinced that it will develop into a full discipline.

Honors and Awards: None

Publications: Di Chiro, G., Timins, E. L., Jones, A. E., Johnston, G. S., Hammock, M. K., Swann, S. J.: Radionuclide Scanning and Microangiography of Evolving and Completed Brain Infarction in Monkeys. Neurology (In Press)

Serial No. NDS(I)-73 SN/NR 2008(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Supine Myelography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Eugene L. Timins, M.D.
H. E. Fillinger, M.D.

Cooperating Units: Department of Neurosurgery, George Washington University Medical Center, Washington, D.C.

Medical Examiner's Office, City of Philadelphia, Department of Public Health, Philadelphia, Pennsylvania

Diagnostic Radiology Department, Clinical Center, NIH

Laboratory of Pathology, National Cancer Institute, NIH

Man Years:

Total:	.2
Professional:	.2
Others:	.0

Project Description:

Objectives: The overwhelming majority of non-hydrosoluble X-ray opaque contrast medium (Pantopaque) myelographies are carried out with the patient in the prone position. For various technical reasons the examination with the patient in supine position is not routinely performed. Through the years we have become more and more convinced that for an accurate appraisal of the spinal canal and its content supine myelography is indispensable. We have collected a large number of cases in which the pathologic lesion (arteriovenous malformation, tumor, arachnoidal cyst, Chiari malformation) was demonstrated only or better in the supine myelograms. We have, therefore, decided to investigate systematically the normal and abnormal radiographic anatomy recognizable in the supine myelograms, and to assess the relative value of the supine versus the prone roentgenograms.

Methods Employed: Three investigation routes were followed:

1) We have collected as many cases as possible of clinical examinations

in which both prone and supine myelograms were available. We have made a point that in each meylographic procedure which we have supervised, both prone and supine films have been taken. In this respect, we have used comparatively large amounts of X-ray contrast medium (from a minimum of 12 cc to 24 cc and more).

2) Prone and supine postmortem myelograms have been carried out using total substitution of the spinal CSF with X-ray contrast medium (up to 100 cc of Pantopaque were employed for this purpose).

3) Anatomical dissection of the posterior subarachnoidal space was carried out in 43 spinal cords removed from cadavers at the office of the medical examiner of a large American city. Cords were removed at random from subjects who ranged in age from 6 to 81. All deaths were due to assault or were suspicious enough in etiology as to require an autopsy as prescribed by law; none of the subjects studied were known, however, to have died of spinal cord injury or to have had a history of gross spinal cord dysfunction prior to death. The spinal cord was removed through an anterior approach, and care was taken to preserve the dura. The cords were then fixed for periods of from 3 days to 3 weeks by hanging them from the cauda equina into a tall vat of 10% formalin.

Fixed cords were then dissected from the upper cervical region downward under a 2X to 10X power dissecting microscope. Using microsurgical instruments, an incision was made through the dura about 3 mm to either side of the posterior midline of the cord. A second incision was then made 4 mm (approximately) farther lateralward from the initial incisions. The lateral strips of dura were then removed, leaving an elongated "island" of dura overlying the midline posterior structures. After observation of the subdural space symmetrical incisions were made through the outer arachnoidal membrane about 5 mm on either side of the midline. The cut edges of the arachnoidal membrane were lifted and retracted upward and medially. Using this dissection methodology careful observations of the posterior meningeal spaces were possible. Pictures were taken using a Nikon "F" camera with a 250 mm medical Nikkor lens with 1X and 2X magnification. Lighting was provided by a strobe light to insure that the fine leptomeningeal trabeculations and compartments could be elucidated, and the subtle distinctions in coloration, detail, and depth appreciated. Notes of our observations were also compiled at the time of dissection.

Major Findings: The elements which have been analyzed in the posterior subarachnoidal space are the septum posticum, the trabeculae located laterally between the septum posticum and the denticulate ligaments, and the occasional leptomeningeal diverticula.

The septum posticum is the midline membrane extending between the outer surface of the pia and the layers of the arachnoid membrane. The septum, posticum, however, is not as distinct and clear an anatomical structure as the denticulate ligaments. This is one of the reasons why many neurosurgeons,

although they may have had significant experience in the surgery of the spinal cord, are unaware of the existence of this midline membranous structure. In fact, even in our dissections, while the denticulate ligaments stood out always as robust and conspicuous structures, the septum posticum was a less evident formation. Also, while the supporting beams of the denticulate ligaments reach the dura at more or less regular, although not strictly metameric, intervals, the septum posticum never does. A distinct, subdural midline posterior septum has been observed by us quite frequently in the cervical segment--31 out of 43 cases--and less frequently in the thoracic and lumbar areas. This subdural membrane does not, however, appear to be an extension of or related to the septum posticum.

In the upper cervical segment the septum posticum is only seldom clearly recognizable as a continuous membrane. In most instances the cervical equivalent of the septum posticum is represented by interrupted and irregular bands which become denser, and actually unite as a distinct membrane beginning at the lower part of the cervical enlargement. Behind the thoracic cord and in the lumbar area, the septum is almost always a clearly individualized structure, although often it presents irregular perforations. At the conus the posterior septum comes to an abrupt end, and below the conus midline leptomeningeal bands which could be considered as part of a septum are seldom found. The septum appears thicker as the age of the donor increases, and this is at variance with the destiny of the trabeculae which will be discussed below.

The trabeculae located between the septum posticum and the denticulate ligaments are more numerous paracentrally, close to the septum posticum rather than more laterally, close to the denticulate ligaments. The trabeculae are generally sparse in the cervical area as compared to the thoracic and lumbar segments. A distinct relationship with age does exist, the trabeculae being more numerous and sizable in the younger and fewer and thinner in the older patients. The arrangement of the trabeculae is haphazard, with a random distribution in different patients and in various segments of the same patient's spine. In some instances the trabeculae may coalesce forming compartments resembling palisades or crypts. Quite often bundles of trabeculae join and merge medially with the septum posticum or laterally with the denticulate ligaments.

Only two distinct leptomeningeal diverticula were observed in this material. Both were found in older individuals, 61 and 57 years old respectively, and were located in the thoracic segment. One of the diverticular sacs was covered by a calcified plaque. Parenthetically plaques of calcifications of the arachnoid membrane, not connected with diverticula, are frequently noted in patients above 20 years old.

How do the above described anatomical peculiarities and the distribution pattern of the leptomeningeal structure in the posterior subarachnoid space affect the pattern of the supine myelogram? In the cervical region the antero-posterior myelogram with the patient on his back does not show peculiarities which clearly distinguish it from the prone cervical myelogram. The

supine across-the-table lateral view is, of course, different from the prone, particularly for the collection of Pantopaque in the declive (lowermost) section of the cisterna magna located below the foramen magnum. Also the ligamenta flava do indent the posterior aspect of the middle and lower cervical Pantopaque column, particularly if the patient's neck is hyperextended (dorsiflexion). The changes in the posterior subarachnoidal space connected with the dynamics of the highly movable cervical segment have been examined in detail by other authors. The thoracic and thoracolumbar antero-posterior myelogram is in most instances quite characteristic with its longitudinal, irregular midline defect, which may change its appearance from metamere to metamere. The defect varies in size and its lateral contours may be straight or oblique but seldom rounded. In the lateral view of the thoracic and thoracolumbar segments the posterior contour of the Pantopaque column appears irregular. This is in contrast to the anterior contour which runs smoothly against the vertebral bodies and intervertebral discs.

An obvious problem with the interpretation of the supine myelograms is the distinction of the normal from possible abnormalities. One will have to learn how to differentiate the defects created by the septum posticum and other arachnoidal septa from those due to other normal structures such as the cord itself and vessels of the cord.

Significance to Bio-Medical Research and the Program of the Institute: Our better understanding of the radiographic anatomy of the posterior part of the spinal canal permits us to use advantageously the supine myelograms. This in turn, should increase our ability to diagnose with precision the various forms of cord and spine pathology.

Proposed Course of Project: Project terminated.

Honors and Awards: None

Publications: Di Chiro, G., and Timins, E. L.: Supine Myelography and the Septum Posticum. RADIOLOGY (In Press)

Serial No. NDS(I)-73 SN/OC 2009(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neurological Basis of Acupuncture-Analgesia

Previous Serial No.: Same

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Herbert Lansdell, Ph.D.; Daniel Ahlberg, M.D.; Melvin Gravitz, Ph.D.; Anthony Bak; Ching-Yuan Ting (Chinese traditional Medical Doctor and Acupuncturist) and Dolores Blessing, R.N.

Cooperating Units: Laboratory of Neural Control, Laboratory of Neurophysiology, Veterinary Resources Branch, Division of Research Services.

Man Years:

Total:	1.0
Professional:	0.6
Other:	0.4

Project Description:

Objective: To investigate the efficacy of the so-called acupuncture-analgesia and the possible change in body function following acupuncture.

Methods Employed: Fifteen normal volunteers are to be invited to participate in this project. The volunteers are informed of all the procedures to be carried out, sign an informed consent and have the right to discontinue their participation in the study. They are then subject to a complete physical, neurological and psychological examination. The psychological examination, which includes the MMPI and one or two intellectual tests and Hingel's test, is to provide objective criteria in the selection of the volunteers. The psychological examination lasts for 4-5 hours.

The selected volunteer comes back a few days later for physiological studies. These consist of EKG, B.P., respiratory movement, EEG, averaging evoked potentials, pain threshold, blood examination, etc. in response to acupuncture. This study lasts for approximately 5 hours. A few days later, the volunteer returns for similar studies and receives, instead of acupuncture, hypnosis. Another few days later, the volunteer again returns for placebo-acupuncture. The sessions for acupuncture, hypnosis and placebo-acupuncture are held in randomized or counter-balance sequence. Throughout the study, the

data are recorded in a polygraph and stored in magnetic tapes. The tapes are to be analyzed with the aid of a computer.

Major Findings: As this report is written, the recordings from eight volunteers have been completed. The preliminary examination records seem to suggest that the pain threshold does not change under the influence of acupuncture. However, no decisive statement can be made at this time before all the data are analyzed.

Significance to Biomedical Research and the Program of the Institute: Acupuncture-anesthesia or acupuncture-analgesia and acupuncture for treatment of avariety of diseases has aroused a great deal of interest, yet nobody knows how and why it works if it really works. It appears that NIH is obligated to evaluate the efficacy and usefulness of this procedure and to assess the limitation and risks involved in acupuncture.

Proposed Course of Project: If acupuncture-analgesia is proven to be a form of hypnosis, the project should be terminated. If it is not, it should be further investigated with greater enthusiasm and efforts.

Honors and Awards: None

Publications:

Li, C.-L.: A brief outline of Chinese medical history with particular reference to acupuncture. Perspect. Biol. Med. (in Press)

Serial No. NDS(I)-73 SN/OC 2010(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanisms of Pain

Previous Serial Number: Same

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Anthony Bak and Daniel Ahlberg, M.D.

Cooperating Units: Laboratory of Neurophysiology, NINDS

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objective: To study the physiological mechanisms of pain and changes in pain threshold by other sensory inputs.

Methods Employed: The experiment is carried out in cats. The saphenous nerve is exposed and bathed in liquid paraffin, the temperature of which is regulated with a thermistor. A pair of electrodes is placed over the distal end and another over the proximal end of the nerve. Between the two pairs of electrodes the nerve is grounded through a capacitor. Electrical stimuli of different intensities, polarities, waveforms, durations and frequencies are applied to the nerve through one pair of electrodes while recordings are made through the other pair.

Major Findings: Stimulation with Haver-sine-wave pulses blocked the A-fiber responses of the nerve but evoked the C-fiber responses. Further, the responses of the C-fibers, under this arrangement, persisted for a long period of time without changes in their latency, amplitude and configuration.

Significance to Biomedical Research and the Program of the Institute: The C-fiber responses obtained by stimulating a mixed nerve by Haver-sine-wave pulses as described above is a significant improvement compared with the method previously reported (Koslow, Bak and Li, 1973). As it is generally known that pain is one of the major defense mechanisms in the body and that C-fibers are a factor of mediating pain sensation, the present experimental

model can facilitate the study of the mechanisms of pain.

Proposed Course of Project: The present study is to be continued with microelectrode recordings from various levels of the nervous system to identify the neurons which respond to C-fiber stimulation. The response of these neurons will be studied under the influence of stimulation applied to other nerve fibers and under the influence of various pharmacological agents.

Honors and Awards: None

Publications:

Koslow, M., Bak, A. and Li, C. L.: C-fiber excitability in the cat. Exp. Neurol. 41: 735-753, 1973.

Serial No. NDS(I)-74 SN/NR 2072(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Blood-Brain Barrier and Radiographic Contrast Media

Previous Serial Number: None

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Norman D. Peters, M.D.
Mary K. Hammock, M.D.
Eugene L. Timins, M.D.
Milton W. Brightman, Ph.D.
Kenneth Earle, M.D.
Jean R. L. Herdt, M.D.
John Fox, M.D.
Torsten Almeń, M.D.

Cooperating Units: Department of Neurosurgery, George Washington University, Medical School, Washington, D.C.

Section on Neurocytology, LNNS, NINDS, NIH

Armed Forces Institute of Pathology, Washington, D.C.

Diagnostic Radiology Department, Clinical Center, NIH

Department of Diagnostic Radiology, Malmö General Hospital, Malmö, Sweden

Man Years:
Total: .2
Professional: .2
Others: .0

Project Description:

Objectives: Neurological complications after intravascular (angiography) and intrathecal or intraventricular (myelography, ventriculography) introduction of hydrosoluble radiographic contrast media (various types of iodinated compounds including the recently developed polimeric, ion-balanced, and non-ionic) still represent a real and serious risk in the neuroradiological procedures. In turn, this inherent danger constitutes a deterrent which prevents the performance of frequently needed procedures.

There is general agreement that the critical phenomenon in the chain of events leading to neurotoxicity by radiographic contrast media is the passage of the media from blood, respectively from CSF, into the neural tissue. This passage is probably determined, among other factors, by the osmotic shrinkage of the cerebro-vascular endothelial cells and opening of the so-called tight junctions.

We have initiated a long range research project to evaluate the disruption of the Blood Brain Barrier (BBB) and Blood-CSF Barrier (BCSFB) by radiographic contrast media either introduced either into the blood stream or within the CSF cavities.

Methods Employed: Our attention will be focused on the monkeys spinal cord. This model has many advantages. We have had a long experience with selective contrast media introduction within the cord vessels (arteriography) and in the CSF around the cord (myelography). Arteriograms and myelograms will be performed in monkeys using the various types of contrast media available. The procedures will be carried out to the threshold of cord damage and beyond--to the point of first production of actual cord damage. Evoked spinal cord potentials will be used to determine the point of early cord damage. Appropriate perfusions will enable us to study by electron-microscopy (EM) the damage of the tight junctions.

Major Findings: We have established the threshold of production of post-angiographic paraplegia in the monkeys using one type of radiographic contrast medium.

Significance to Bio-Medical Research and the Program of the Institute: Increase our understanding of the neurological complications connected with use of radiographic contrast media. Establish the point of reversibility--respectively irreversibility--of these complications.

Proposed Course of Project: To follow our research according to the above described guidelines.

Honors and Awards: None

Publications: Di Chiro, G.: Unintentional Spinal Cord Arteriography: A Warning. RADIOLOGY (In Press)

Serial No. NDS(I)-74 SN/NR 2073(c)
1. Surgical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Computerized Axial Tomography of Hindbrain and Spinal Cord

Previous Serial Number: None

Principal Investigator: Giovanni Di Chiro, M.D.

Other Investigators: Robert Ledley, Ph.D
Dieter Schellinger, M.D.
Alfred J. Luessenhop, M.D.

Cooperating Units: Department of Radiology, Georgetown University Medical School, Washington, D.C.

Department of Neurosurgery, Georgetown University Medical School, Washington, D.C.

Man Years:

Total:	.1
Professional:	.1
Others:	.0

Project Description:

Objectives: The new radiographic method, Computerized Axial Tomography (C.A.T.) of the brain, by means of the British developed EMI-Scanner, is revolutionizing the diagnostic methodologies for many types of brain lesions: tumors, bleedings, infarctions, hydrocephalus. The EMI-Scanner, however, does not permit us--because of some technical construction features--to study the postero-inferior part of the posterior fossa, the base of the skull, and the cranio-cervical junction. Thus, the hindbrain cannot be evaluated by means of the EMI-Scanner. Also, this device does not allow us to study the spine and its content (and hence, the spinal cord). A new equipment for C.A.T., the ACTA-Scanner, very recently developed by Dr. R. Ledley of the Georgetown University Medical School, does permit the evaluation of: 1) the postero-inferior part of the posterior fossa; 2) the base of the skull; 3) the cranio-cervical junction; 4) the hindbrain; 5) the cervical spine and cord. From our preliminary experience, it is not clear yet whether or not the content (particularly the cord) of the thoracic and thoracolumbar segments of the spine will be within the resolution capabilities of the ACTA-Scanner.

Methods Employed: Patients with various types of hindbrain and spinal

cord pathology are studied with the ACTA-Scanner.

Major Findings: A first major breakthrough is that we have been able to demonstrate the central cavity of one (possibly 2) case of syringomelia.

Significance to Bio-Medical Research and the Program of the Institute: The importance of diagnosing pathology of the hindbrain and the cervical cord by means of a non invasive and innocuous technique is self evident.

Proposed Course of Project: Gather further clinical experience with the ACTA-Scanner in hindbrain and spinal cord pathology of various types (cystic cavities, tumors, bleedings, infarctions, spine and cord injuries, spondylotic myelopathy, congenital anomalies).

Honors and Awards: None

Publications: None

ANNUAL REPORT

July 1, 1973 through June 30, 1974

Central Nervous System Studies Laboratory

National Institute of Neurological Diseases and Stroke

National Institutes of Health

The Central Nervous System Studies Laboratory is an outgrowth of the Study of Child Growth and Development and Disease Patterns in Primitive Cultures, under the direction of D. Carleton Gajdusek, M.D. It was the parent organization that gave rise to the discovery of kuru, a heredofamilial subacute progressive degenerative disease of the central nervous system of the Fore people, in the Eastern Highlands of Papua New Guinea, and led to the demonstration that this disease is caused by a most unusual and atypical virus. In addition, the successful transmission of kuru provided the necessary techniques for the subsequent demonstration of a viral etiology for the Creutzfeldt-Jakob type of sporadic and familial presenile dementia, the transmissibility of a case of familial Alzheimer's disease, and the development of spongiform encephalopathy in animals inoculated with specimens obtained from a human dying with the diagnosis of Köhlmeier-Degos disease, associated with minimal status spongiosis of gray matter of the brain, as seen in kuru and Creutzfeldt-Jakob disease.

Initially, the Study of Child Growth and Development and Disease Patterns in Primitive Cultures was an all-encompassing program that included direct operation of laboratory studies on a wide variety of conventional viruses that included the ecology and seroepidemiology of arboviruses in remote areas of the world, the evaluation of the efficacy and long-term sequellae following the use of live attenuated measles virus vaccine in previously unexposed, immunologically virgin populations, and the definitive analysis and identification of the antigenic strain of influenza that was involved in the 1918-1919 world pandemic of this latter disease.

Concomitantly, the Study of Child Growth and Development and Disease Patterns in Primitive Cultures was engrossed in the investigation of endemic goiter in the Central Highlands of Western New Guinea, as well as on patterns of delayed puberty and slow growth rates. Congenital mental defects, endemic cretinism and familial periodic paralysis were also being investigated. Such studies led to the discovery of new genetic factors of haptoglobins and hemoglobins, and the definition of their biochemical structure.

With greater emphasis being placed on studies to determine the nature of the viruses causing subacute encephalopathies, i.e. kuru, Creutzfeldt-Jakob disease and scrapie, and with intensified efforts to demonstrate infection as the etiology of multiple sclerosis, amyotrophic lateral sclerosis and a wide variety of other central nervous system diseases, it became obvious to the director of the program that the laboratory had developed into two mutually

dependent and closely interrelated projects which diverged at their technical extremes into very different disciplines. However, it is significant to note that the development and maturation of both projects resulted from cross-fertilization of each other since their origin and that the second has grown from the first.

Although the two projects, each composed of several sub-sections, differ mainly in their technical extremes, they are served by the same investigators who function as a team. These scientists derive their creative stimulus, dedication and enthusiasm to a great extent from the atypical materials presented and the diverse, frequently unconventional, approaches phrased by the two projects. The two projects which constitute the Central Nervous System Studies Laboratory are:

Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

and

Chronic Central Nervous System Disease Studies: Study of Slow, Latent and Temperate Virus Infections

Principle Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.

Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

A basic understanding of human biology, including in-depth data on neurobiology, immunological 'original sin' and behavioral patterns have thus far been best obtained in the study of rapidly vanishing primitive societies in the remote areas of the world. Our laboratory research and that of our numerous collaborating investigators is directed towards problems of the neurological development and learning patterns in children in diverse cultural experiments in the human condition found in these isolated primtive populations. Laboratory studies on human biology, genetics and associated molecular biology, on immunology, virology and biochemistry, and on the biological adaptation to diverse and extreme environmental conditions, have all been directed at solving problems which have been carefully chosen from small isolated bands still lviing in the primitive situation in which these problems may be more appropriately studied than in larger civilized societies. Nutritional studies, studies on reproduction and fertility, on neuroendocrine influences on age of sexual maturation and aging, and on studies of the selective advantage and establishment of genetic polymorphisms are all under way. The unusual and odd alternatives of employing the cnetral nervous system in its higher cerebral function of patterns of learning, language, cognitive styles, computation (number sense and calculation without a number system), and psychosexual culturally modified behavior, are providing data on alternative forms of possible neurological functioning for man of which we would remain unaware and for moral, ethical and political reasons be unable to produce or investigate in the clinic or laboratory, once the natural cultural experiments in primitive human population isolates had been finally amalgamated into the modern civi-

lized cultural veneer which is now imposed on almost all members of the community of man.

Growth and development studies in primitive cultures have yielded evidence for delayed puberty and slow growth rates in certain peoples, particularly the short-statured populations in the highlands of New Guinea. Preliminary evidence suggests high levels of pituitary growth hormone in such populations from the pituitary glands obtained at autopsy, and in serum specimens. The growth rate seems to be proportional and age of puberty inversely proportional to mean adult stature. Congenital and mental defect and a wide range of neurological problems associated with the most severe foci of endemic goiter and cretinism in the world as a result of severe iodine deficiency, in the highlands of Western New Guinea, are under further investigation. These are contrasted with enclaves of familial goiter and cretinism associated with thyroid dysfunction in the coastal populations on small islands. The frequent but irregular association of familial deaf-mutism, with goiter and cretinism, is under study.

The discovery of new genetic factors, including haptoglobins, hemoglobins and red cell enzyme pleomorphisms, and the elucidation of their biochemical structure and their later use as markers in human population genetic studies, have been byproducts of these investigations. The discovery of immunologically virgin populations, without exposure to respiratory and enteroviruses which are ubiquitous in the civilized world, has permitted fundamental investigation of the immune response in man, investigations possible nowhere else on the persistence of the immune response after natural measles infection and live attenuated measles virus immunization in the absence of circulating virus, yielding data important to the diagnosis and understanding of delayed slow measles encephalitis (subacute sclerosing panencephalitis, or SSPE), and to establishing the serological identity of the agent of the 1918 influenza pandemic to aid in the genetic-historical elucidation of the serial mutation of influenza virus. By virtue of limited travel during their entire lifetime and exposure to their natural ecology, members of primitive groups serve as unequaled sentinel populations for revealing the focal microbial agents that infect man in his environment. Thus, for infections ranging from Chagas disease and toxoplasmosis to arbovirus infections, primitive groups offer unusually fruitful samples for investigation. On toxoplasmosis, filariasis, yaws and malaria, as well as on arbovirus encephalitides, we have studies in progress.

With the current interest in papovaviruses (SV40, J.C., BK) latent in man, as the cause of progressive multifocal leucoencephalopathy, the presence and pattern of acquisition of antibodies to these in isolated primitive groups, with no contact with primates or vaccines, help settle the problem of the origin of these infections in man. In most of the island populations groups examined, representing over a thousand serum specimens from Micronesia, Melanesia, Australian aborigines, South American Indians, Alaskan Eskimos, isolated Icelandic villages, and diverse groups from the Middle East, we have confirmed a high level of exposure to BK virus in the avsence of association with overt disease, with prevalence ranging from 60% to 90%. Furthermore, SV40 antibodies occur almost exclusively in individuals with high levels of antibody to BK virus, suggesting far more cross-reactivity than has been previously

suppressed. Similar antibody acquisition patterns for cytomegalovirus, Epstein-Barr virus, and herpesviruses types 1 and 2 and other simian--including foamy viruses--are also being studied in these idolates, to add to our knowledge of their biological role. We have, for instance, noted the very early acquisition of cytomegalovirus and Epstein-Barr virus antibodies in these isolated groups in some cases with positive titers reaching 100% by mid-childhood.

Our efforts to document the development and neurological patterning in the disappearing primitive cultures has resulted in one of the largest archives of such documentation in the world. The collection and preservation of cinema data from New Guinea, Oceania, Australian aborigines, African aborigines and American Indian groups as they live as hunter-gatherers or primitive hoe and digging stick agriculturists, provides the only such documentation of the life and behavior of man as he lived and evolved for most of his evolutionary history about three million years. Our many and diverse investigations have evolved about the theme of elucidating the medical problems in the cultures of primitive man, or man living in small isolated communities, by carefully selecting such problems of immunological, virological, biochemical, genetic, reproductive biology, endocrinological and nutritional interest--all pertinent to the long term studies of growth, behavior, emotional responses to stress and human biology in these groups.

Potent tools of mathematical population genetics have been used with our massive genetic pleomorphism data on primitive groups for the analyses of the multilocus genetic effects in determining expression of neurological diseases such as kuru, and a similar approach is under way for the transmissible virus dementias. Genetic distance techniques including genetic tree construction and principal components analyses are used.

Our studies in the British Solomon Islands and in New Hebrides and those in the Caroline Islands of Micronesia were greatly expanded last year by the expedition on the Research Vessel Alpha Helix of the Scripps Institution of Oceanography and the support of the National Science Foundation for our medical and population genetic studies in these islands, with a large team of investigators from our own laboratories and those of our close collaborators. Based on the initial field investigations during the expedition, we examined over two thousand islanders for clinical evidence of malaria and by serological examination confirmed the widespread existence of malarial infection with multiple malaria vectors on many of the islands, while others have only single species vectors or are malaria-free. The World Health Organization malaria eridacation programs are already under way in these areas, and our comprehensive results will be invaluable to those involved in these programs. Other studies, involving mycobacterial and fungal skin testing of entire island populations has led to the curious and quite interesting observation that New Hebrides island populations which had received BCG vaccination in previous years had the lowest rate of skin positivity, whereas an unvaccinated Solomon Island population with no clinically apparent tuberculosis had the highest rate of skin positivity.

Laboratory studies are continuing, sorting out and testing the vast medical, genetic and human biologic data collected during the Alpha Helix expedition. We anticipate that it will be some time before all these studies,

initiated during the expedition, are completed. The knowledge we gain from such studies will no doubt, as has been demonstrated in the past, have vast impact on medical science.

Chronic Central Nervous System Disease Studies: Study of Slow, Latent and Temperate Virus Infections

The discovery of an intense foci of chronic degenerative neurological disease, kuru, in a remote and isolated area of Papua New Guinea led to the establishment for the first time that a subacute degenerative disease of the central nervous system of man is caused by a highly pathogenic virus. It was the discovery of kuru that prompted the NINDS to establish a Laboratory for the Study of Slow, Latent and Temperate Virus Infections, with a responsibility for conducting research studies not only on kuru, but on multiple sclerosis, amyotrophic lateral sclerosis, presenile and senile dementias of the sporadic and familial types, and other central nervous system diseases whose clinical signs, histological lesions and epidemiological pattern suggested inclusion into the group now referred to as "slow virus infections."

The slow virus theory as the etiological factor in diseases of the central nervous system was a direct outgrowth of our attempts to solve the riddle of an epidemic of kuru in the Fore people of Papua New Guinea. This exotic model was chosen for such intensive study for the obvious implications its solution would have to other central nervous system degenerative diseases of man which it resembled in basic pathologic lesions and even to the degenerative processes. Notably among these diseases was the presenile dementia of Creutzfeldt-Jakob-type and the familial occurrence of Alzheimer's disease. Microbiology had already given solution to the etiology of most acute infections of the CNS, and fungi and bacteria had been incriminated in important chronic disorders of the nervous system, such as torula and tuberculous meningitis. Neurology was left with a wide range of subacute and chronic affections of the central nervous system of unknown etiology. Some of these diseases, still listed as idiopathic, are among the most prevalent afflictions of the CNS. Many others with familial patterns of occurrence still do not have their basic pathogenesis or underlying metabolic defect elucidated, although we tend to think of them as genetically mediated. It was our conviction that if any of these diseases fell into the category of slow, latent or temperate virus infections, if even a single one of these many syndromes of unknown etiology could be traced to a virus or virus-gene interaction, we would make significant advances in our understanding of diseases of the human brain. We felt that the time had come for virologists, neurologists and neuropathologists together renew their attack on age old, still unsolved problems, bringing modern methods of microbiology and immunology to bear once again on these mysteries with the powerful new techniques of tissue culture, immunofluorescence and electron microscopy to aid them and the new concepts and understanding of temperate infections, virus-gene intereaction, virus-host cell membrane interaction, immunopathological mechanisms, and "slow infections" to direct their thinking.

The problem of the long survival or persistence of chronic, latent,

masked, recurrent and long incubation infections, and of the carrier state were all well known phenomena already studied at the dawn of virology. As we pointed out in 1964 "these are neither new ideas nor new problems, nor do they necessarily involve new viruses". The accuracy of this perspective in virology was proven following the successful transmission of kuru with the isolation and identification of defective measles virus as the etiological agent of subacute sclerosing panencephalitis (SSPE) in children and young adults, the idolation and identification of J.C. and SV40 papovaviruses from the brains of patients with progressive multifocal leucoencephalopathy (PML), the demonstration of nucleocapsids and mature virions in the brain of patients with multiple sclerosis, and in some instances the demonstration that specific antibody to measles and, to a lesser extent to vaccinia in the cerebrospinal fluid of some patients with multiple sclerosis, and the detection of virus inclusion bodies in the brain of patients dying of epilepsy partialis continua, or chronic focal epilepsy, with progressive brain pathology.

The choice of studying kuru as the central theme of our program was a wise one. The intense localized phenomenon of the disease offered a possibility of a breakthrough. The design for the program was, and continues to be, studies on the nature and epidemiology of subacute progressive degenerative diseases of the central nervous system and the eluicidation of the causes and pathogenesis in man. In less than a decade these studies have made significant contributions to our understanding of subacute neurological diseases. They have documented the transmissibility of kuru from man to the chimpanzee, without exception. Thus, kuru became the first chronic brain disease of man to be shown to be a 'slow infection' with viral etiology. During the last two years the experimental host range for this disease has been expanded to include several species of Old World and New World monkeys (rhesus, gibbon-ape, squirrel, capuchin, woolly and marmoset). Moreover, recently collected data show that the disease can be associated with remarkably long asymptomatic incubation periods (8½ years in the rhesus monkey), during which pathological lesions, however, are slowly accumulating within the brain, primarily in gray matter. Pathogenesis studies show the virus of kuru is present not only in tissues of the central nervous system, but in visceral tissues as well. Nor it is necessary to assault the brain in order to induce experimental disease, since we have now shown transmissibility by single and multiple routes of peripheral inoculation (sc, ip, im). During the period covered by this report we have expanded our studies to include purification of the kuru virus by physical and chemical procedures, in an attempt to better define the biological characteristics of the agent. It is now established that the most significant advances have occurred primarily on the periphery of the spectrum of the degenerative central nervous system diseases under study.

Techniques developed during the early studies of kuru led us to the remarkable demonstration for the first time that presenile dementias of the Creutzfeldt-Jakob type, which are not ethnically nor geographically restricted, are caused by a similar virus. The further pursuit of this lead has made possible the definition of the subacute spongiform virus encephalopathies as a group of related diseases of man (kuru and C-J disease) and animals (scrapie of sheep, and transmissible mink encephalopathy). During the past two years further study of patients with progressive dementia has provided the surpri-

sing demonstration that even the heredofamilial form of C-J disease is transmissible and of virus etiology. Although most cases are sporadic there are families in which the disease has occurred in siblings, parents, and close maternal and/or paternal relatives over several generations. From two such families the disease has now been experimentally transmitted to primates, demonstrating for the first time in man that disease with apparently genetic determination may be caused by a slow virus. Most important has been the demonstration that the virus of C-J disease is presnet not only in the brain, but in the liver and lymph nodes of a patient that died with familial disease. The onset of clinical signs and the acute progression of the disease in a patient seventeen months following transplantation of a cornea from a donor who died with Creutzfeldt-Jakob disease, and the occurrence of the disease in a neurosurgeon, are of concern. This has added impetus to our already extensive studies of Huntington's chorea, Alzheimer's and Pick's diseases, parkinsonism-dementia and even senile dementia. These investigations have so broadened the picture of C-J disease that we now must refer to transmissible virus dementias. This is necessary, since the diseases in patients with subacute or chronic dementia whose clinical and pathological diagnoses have been Alzheimer's disease and papulosis atrophicans maligna of Degos, have been transmitted to primates as subacute spongiform encephalopathies. Thus, the trail from kuru to C-J disease now embraces studies of presenile and senile dementias of all sorts, including "ementia praecox", the organic brain disease associated with late uncontrolled schizophrenia.

The demonstration that non-inflammatory chronic degenerative, even heredofamilial central nervous system disorders, could be of virus etiology, greatly stimulates the search for possible virus etiology in the demyelinating and sclerosing CNS diseases and chronic or actual encephalitides. The sero-epidemiologic approach to multiple sclerosis with antibody determination in the spinal fluid and serum has led to our conviction that at least some of the patients are suffering from delayed and slow defective virus infections, probably with measles virus as Adams and others have earlier suggested. Intensive studies with whole virus and virus sub-unit antigens of the serial spinal fluid and sera, and gammaglobulin extracted from autopsy brain specimens, for antibody activity is under way. Search for such antigens in brain tissue is in progress.

The two disorders involving white matter, which have been demonstrated to be of slow virus etiology are 1) delayed and slow measles encephalitis (SSPE) and 2) progressive multifocal leucoencephalopathy (PML), from which two papovaviruses, SV40 and J.C. have now been isolated. The large series of brain biopsy and autopsy specimens, sera and spinal fluid we had assembled on these diseases, have materially contributed to their elucidation. It now appears that even certain forms of chronic progressive epilepsy are caused by slowly progressive chronic focal encephalitis. We have in the laboratory an experimental model in rhesus monkeys for this disease, using a strain of tick encephalitis virus (RSSE) which causes, after recovery from acute encephalitis, slowly progressive movement disorders and focal myoclonic epilepsy. Thus, the range of chronic and degenerative central nervous system diseases, which must be further studied from a virological and immunological approach, have been extended.

In an attempt to unmask viruses in these diseases, brain cells have been grown in vitro by both primary explant and trypsinized cell layer techniques from specimens obtained at biopsy and early autopsy. Co-coltivation and cell fusion and BUDR and IUDR induction are major techniques employed. The presence of a virus in these cultures is sought by electron microscopic scanning, staining for inclusion bodies, search for CPE, hemadsorption, fluorescent antibody, interferon production, vurus interference and animal inoculation. The same techniques are applied to surgically sterile tissue obtained from experimental animals affected with kuru, Creutzfeldt-Jakob disease and other slow virus infections. As a by-product to the work, many masked and latent viruses from chimpanzee and monkey hosts have been isolated. These include herpesviruses (including cytomegaloviruses and Epstein-Barr virus) and a wide series of new foamy viruses. The latter foamy viruses are of particular interest in that they are oncornaviruses characterized by the production of a reverse transcriptase, yet they have not caused cell transformation in vitro or tumors in monkeys. It has been shown that they are present in the brain and other organs of most chimpanzees and monkeys. We have recently developed a complement fixation test for studying the seroepidemiology of foamy viruses.

The long maintenance of brain cells, presumably astroglial cells, dedifferentiated on passage to resemble fibroblasts, regularly result in cell lines which propagate indefinitely and show many properties that suggest transformation. More blatant transformation with full loss of contact inhibition has occurred in three episodes with human brain cells: in the first instance from a C-J disease patient's brain tissue with regular presence of a Mason-Phizer monkey virus-like MP-MV oncornavirus in the transformed human brain cells. The other two transformations have occurred in two brain cell lines from C-J patients which transformed with full loss of contact inhibition but without the presence of recognizable virions in the transformed cells. In addition, a transformed cell line from a class II astrocytoma has been established and attempts to demonstrate virions are in progress. These transformations have given further support to the speculation that the slow virus-cell interaction in the degenerative central nervous system diseases may be closely related to the slow virus-cell interaction in cell transformation and carcinogenesis. The use of the term "temperate" in naming our laboratory in 1962 seems to have been prophetic.

A group of atypical viruses unlike any others known to microbiologists appear to cause the subacute spongiform virus encephalopathies. The other CNS degenerations caused by slow viruses are caused by rumbling non-productive, even defective, infection with the more conventional measles, papovaviruses (SV40 and J.C.), rubella virus, cytomegalovirus, herpes simplex virus, adenovirus type 32 and RSSE. The atypical viruses (kuru, C-J disease, scrapie and TME) have unusual resistance to ultraviolet radiation and ionizing radiation, to heat, proteases and nucleases, and to formaldehyde. They are not associated with a recognizable virion on electron microscopic study of infected cells in vivo or in vitro, or concentrated virus preparations (zonal banding). This has led to the speculation that they represent infectious agents, lacking a nucleic acid, perhaps even a self-replicating membrane fragment. A major effort in this laboratory has been and is now being directed toward the molecular biological elucidation of the nature and structure of this group of atypi-

cal viruses. They have been partially purified (fluorocarbon protein precipitation) and successfully concentrated in zonal density banding in the ultracentrifuge, and other physical and chemical properties and EM structure is under study. Recent data show that partial purification of scrapie infected moust brain by fluorocarbon deproteinization provides for highly infectious preparations which, when exposed to ultraviolet light, causes inactivation of the virus in a linear fashion consistent with most conventional viruses. Moreover, zonal density gradient techniques have produced highly infectious fractions of almost pure membrane which when tested for optical density are highly proteinacious and in the range of nucleic acids. The solution of their structure is of immense importance to general microbiology, since they represent a new type of transmissible agent, unlike any of the other agents with which we work in virology. We currently believe that they are composed of very small units of infectious nucleic acid, probably RNA, perhaps under 100,000 daltons, attached by strong binding to fragments of normal plasma membrane, usually without geometrically recognizable packaging. The tight binding to the plasma membrane must confer the unique high U/V resistance and nuclease resistance to the nucleic acid moiety in crude preparations. Experiments are under way to refute or substantiate this view of their structure, which has been suggested by the preliminary studies.

We have recently initiated the study of human brain from patients with Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, kuru, multiple sclerosis, Alzheimer's and normal human brain to determine the possible presence of sequences of viral genomes in these tissues. This involves a search for the presence of reverse transcriptase, as well as hybridization studies, employing purified viral probes to SV40, B.K. and JC papovaviruses, herpesviruses types I and II, and several additional DNA viruses. Collaborative studies in this work are being conducted with investigators at the Scripps Institute and the University of California at San Diego. Finally, it is significant to note that during the second quarter of the fiscal year of this report on the Laboratory of Slow, Latent and Temperate Virus Infections, operations at the Patuxent Wildlife Research Center in Laurel, Maryland, were closed down. In an effort to continue this work with a minimum of interruption, it was necessary to relocate the laboratories in scattered fashion on the main campus of the National Institutes of Health. Our major ape colony, inoculated with a variety of human diseases, were relocated to the Gulf South South Research Institute at New Iberia, Louisiana. A smaller number of apes and the major monkey, and some 15,000 mice and smaller laboratory animals on study, were relocated to a temporary facility at Fort Detrick, Maryland.

Serial No. NDS (I)-65 CNSS 969
1. Intramural Research
2. Central Nervous System Studies Laboratory
3. Bethesda, Maryland

PHS - NIH
Project Report
July 1, 1973 through June 30, 1974

Project Title: Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infections

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.

Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, pre-senile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.

Sub-Project II: Characterization and pathogenesis of kuru virus.

Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia) virus.

Sub-Project IV: Studies on the characterization and nature of the viruses of scrapie, transmissible mink encephalopathy and visna diseases.

Sub-Project V: In vivo and in vitro host range of the subacute spongiform virus encephalopathies.

Sub-Project VI: Isolation and characterization of adenovirus from the urine of chimpanzees.

Sub-Project VII: Fluorescent antibody studies on the intracellular localization and identification of viral antigens in vivo and in vitro in tissues from patients with subacute diseases of the CNS.

Sub-Project VIII: Tissue and cell culture in vitro studies of virus induced slow infections of man and animals.

Sub-Project IX: The simian foamy viruses.

Sub-Project X: Studies on transformed human brain tissue in vitro and characterization of associated virus.

Sub-Project XI: Isolation and characterization of cytomegalovirus from the urine of chimpanzees.

Sub-Project XII: Characterization and identification of new herpes viruses from explant cultures of tissues from sub-human primates.

Sub-Project XIII: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.

Sub-Project XIV: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

Sub-Project XV: Development of serological and immunological test system for use in the study of slow infections of the CNS.

Sub-Project XVI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.

Sub-Project XVII: Animal management and intercurrent diseases in sub-human primates on long-term studies of slow infections.

Sub-Project XVIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.

Other Investigators: David M. Asher, M.D.; Herbert Amyx, D.V.M.; Paul Brown, M.D.; Richard Benfante, M.A.; Eric French, Ph.D.; Louis Hertzberg, M.D.; Judith Meyer; Randy Taylor; Theodore Tsai, M.D. Roger Traub, M.D.; Lon White, M.D.; Monica Lewis, B.S.

Visiting Scientists: Eric French, Ph.D.; Louis Hertzberg, M.D.

Technical Assistants: Alfred Bacote, Lucille Gilbert, Nancy Luber, Joseph Pazdersky, Hubert Saville, Ezra Schafer, Michael Sulima, Leon Vance, Eugene Webster, Harry Winpigler

Cooperative Students: Janie Ensley, Phyllis McDonald, Ann Rudnick, Robert Wismer, Terry Zettelmeyer

Cooperating Units: Michael Alpers, M.D., University of Western Australia, Perth, Australia; Eric French, Ph.D., Division of Animal Health, CSIRO, Victoria, Australia; Robert L. Kirk, M.D., Department of Genetics, Australian National University, Canberra, Australia; Eric Shaw, Ph.D., Red Cross Blood

Transfusion Service, Brisbane, Australia;
Armand Lowenthal, M.D., Laboratory of Neuropathology,
l'Institut Bunge, Antwerp, Belgium;
Theodore Rasmussen, M.D., University of Montreal, Canada;
Helena Libikova, M.D., Institute of Virology, Slovak
Academy of Sciences, Czechoslovakia; Peter M. Daniel,
M.D., Department of Neuropathology, Maudsley Hospital,
London, England; George Dick, M.D., Bland-Sutton
Institute of Pathology, London, England; E.J. Field,
M.D., Medical Research Council, Royal Victoria Infirmary,
Newcastle upon Tyne, England; D. Haig, D.V.M.,
D. Hunter, Ph.D., I.H. Pattison, Animal Research Section,
Compton, England; Francoise Cathala, M.D., Department of
Neurology, Hopital de la Salpetriere, Paris, France;
Volker ter Meulen, M.D., Institut fur Virologie der
Universitat, Wurzburg, Germany; P.A. Palsson, D.V.M.,
Institute for Experimental Pathology, Keldur, Iceland;
R.W. Hornabrook, M.D., Institute of Human Biology, Papua,
New Guinea; Vincent Zigas, M.D., Public Health Department,
Rabaul, New Britain, Papua, New Guinea; Ewa Osetowska,
M.D., Institute of Neuropathology, Polish Academy of
Science, Warsaw, Poland; C. Espana, Ph.D., National Center
for Primate Biology, University of California, Davis,
California; F. Dixon, M.D., M. Oldstone, M.D.,
David Kohn, Ph.D., Scripps Clinic and Research Foundation,
La Jolla, California; Peter W. Lampert, M.D., Department
of Pathology, University of California, La Jolla,
California; Roy L. Walford, M.D., Department of Pathology,
University of California, Los Angeles, California;
Freidrich Deinhardt, M.D., Loren Wolf, M.D., Department of
Microbiology, Presbyterian-St. Luke's Hospital, Chicago,
Illinois; Philip Y. Paterson, M.D., Northwestern
University Medical School, Chicago, Illinois;
Wolfgang Zeman, M.D., A.N. Siakotos, Ph.D., Department of
Neuropathology, Indiana University Medical Center,
Indianapolis, Indiana; William Greer, D.V.M., Gulf South
Research Institute, New Iberia, Louisiana; Robert Herndon,
M.D., Richard T. Johnson, M.D., Guy McKhann, M.D.,
William Narayan, M.D., Neil Nathanson, M.D.,
Leslie Weiner, M.D., Keerti Shah, Ph.D., Johns Hopkins
Medical Center, Baltimore, Maryland; Theodore Diener,
Ph.D., Department of Plant Biology, U.S. Department of
Agriculture, Beltsville, Maryland; Paul Albrecht, M.D.,
Lewellys F. Barker, M.D., Hope E. Hopps, J. Anthony Morris,
Ph.D., Bureau of Biologics, FDA, Bethesda, Maryland;
Samuel Baron, M.D., Hilton Levy, M.D., NIAID, NIH,
Bethesda, Maryland; Paul MacLean, M.D., NIMH, NIH,
Bethesda, Maryland; Jacob Brody, M.D., King Engel, M.D.,
David A. Fuccillo, M.D., Luiz Horta-Barbosa, M.D.,
John Sever, M.D., NINDS, NIH, Bethesda, Maryland;

David Poskanzer, M.D., Massachusetts General Hospital, Boston, Massachusetts; W. Hadlow, D.V.M., Rocky Mountain Laboratory, NIAID, NIH, Hamilton, Montana; Robert Ledeen, M.D., Department of Neurology, Albert Einstein College of Medicine, Bronx, New York; Irving Cooper, M.D., J. McKendree Waltz, M.D., Department of Neurology, St. Barnabas Hospital, New York, New York; Donald Harter, M.D., Richard L. Masland, M.D., Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York; R. Katzman, M.D., Yeshiva University, Gerald Ward, D.V.M., Otisville, New York; R. Carp, Ph.D., Halldor Thormar, Ph.D., Institute for Basic Research in Mental Retardation, Staten Island, New York; John Griffith, M.D., David Lang, M.D., Department of Pediatrics, Duke University Medical Center, Durham, North Carolina; Hilary Koprowski, M.D., Wistar Institute, Philadelphia, Pennsylvania; J. Hourrigan, D.V.M., Animal Research Section, U.S. Department of Agriculture, Washington, D.C.; K. Earle, M.D., Sam Chou, M.D., Department of Neuropathology, West Virginia University Medical Center, Morgantown, West Virginia; A.M. Gardashyan, M.D., Department of Immunology and Oncology, Gamelaya Institute, Moscow, U.S.S.R.; V.I. Il'yenko, M.D., All-union Research Institute of Influenza, Leningrad, U.S.S.R.; Mrs. Elisabeth Beck, Max-Planck-Institut fur Hirnforschung Neuropathologische Abteilung, Frankfurt, Germany; Klaus Mannweiler, M.D., Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Hamburg, Germany; Gertrude Henle, M.D., Werner Henle, M.D., Children's Hospital, Philadelphia, Pennsylvania; William Schoene, M.D., Peter Bent Brigham Hospital, Boston, Massachusetts; F. Seitelberger, M.D., Neurologisches Institut der Universitat Wien, Vienna, Austria; Raymond Latarjet, Foundation Curie-Institut du Radium, Paris, France.

Administrative: Marion Poms, Linda Poole, La-Donna Tavel

Man Year:

Total	:	38
Professional	:	26
Others	:	12

Publications: Yes, pages 55 through 59

Project Description: The Central Nervous System Studies Laboratory, under direction of the co-principal investigators, has as one of its two projects an integrated team study on Slow, Latent, Temperate, Masked, Persistent and Chronic Diseases of Man and Animals, with particular emphasis on those affecting the nervous system. The major diseases under study are: kuru, Creutzfeldt-Jakob disease, familial Alzheimers, and other presenile and senile degenerative processes affecting man, Pick's, Alper's, Huntington's chorea, epilepsia partialis continua, multiple sclerosis, amyotrophic lateral sclerosis, Parkinsonism-dementia, progressive supranuclear palsy, subacute sclerosing panencephalitis (SSPE), progressive multifocal leucoencephalopathy, Schilder's disease, chronic encephalitis, transmissible papulosis atrophicans of Degos, dementia praecox associated with late uncontrolled schizophrenia. As may be noted in the preamble to this report there are 18 subprojects that make up the scope of the work.

Objectives: The major objectives established in 1961 have not substantially changed. They remain as follows: to elicit the etiology of subacute progressive degenerative diseases of the nervous system of man and animals; to study the pathogenesis of these diseases, their natural method of spread, and their seroepidemiology; to determine the role of the genetic mechanism of the host as it relates to resistance and susceptibility to disease; to determine the natural and experimental host range; to determine the nature of the viruses isolated from naturally and experimentally infected hosts through studies on the physical, chemical, biological, immunological, histochemical, histopathological and genetic mapping properties of the agents causing these diseases; to study the mechanisms of helper and hinderer viruses that may be involved in the pathogenesis of these diseases; to elicit immunopathogenic mechanisms involved, including neoantigens at the cell surface.

Methods Employed: The methods employed in these studies, with necessary alterations to meet the needs of specific problems encountered, remain essentially the same as described in previous reports. The utilization of techniques developed in this program for the isolation and characterization of "atypical" viruses, ubiquitous viruses and other infectious agents from human tissues and animal tissues, particularly from the nervous system of the affected host have been described. Such techniques include inoculation and long-term holding of higher sub-human primates and monkeys, domestic animals and avian hosts and a wide variety of smaller mammalian and avian species in the laboratory; development, establishment and utilization of *in vitro* tissue and cell culture systems from naturally affected humans and animals in which diseases have been induced as well as the utilization of primary and continuous cell lines from a wide variety of human, animal and avian tissues for rescuing viruses; utilization of biophysical, biochemical, histological, histochemical, immunological and immunochemical techniques in an effort to characterize isolates or provide markers for the *in vivo* detection of latent/persistent defective viruses. Contractural phases of this work are being conducted at:

Gulf South Research Institute, New Iberia, Louisiana	491,511.00
Litton Bionetics, Inc., Frederick, Maryland	154,972.00

Public Health Research Institute of New York, Otisville	116,588.00
National Center for Primate Biology, University of California, Davis	40,000.00
Max-Planck Institute for Neuropathology, Frankfurt, Germany	13,050.00
Department of Pathology, University of California, San Diego	4,450.00
Department of Virology, Hopital Salpetriere, Paris, France	3,000.00
Department of Microbiology, University of Western Australia, Perth	2,500.00

This project is part of the Central Nervous System Studies Laboratory (see Project Report Serial No. NDS (I)-1282 (I-XI).

Major Findings: These studies have established infection as the etiology for a number of subacute progressive degenerative diseases of the nervous system of man and animals that include kuru, Creutzfeldt-Jakob disease, familial Alzheimers with spongy degeneration of the brain, and spongiform encephalopathy from a patient with Köhlmeier-Degos disease. The data collected have provided the information necessary to classify slow infections of the nervous system into two broad categories based on the nature of the disease and the physical, biological and immunological properties of the viruses which cause them: (1) "atypical" diseases caused by highly unconventional viruses which form a new group of infectious agents and which we have designated the subacute spongiform virus encephalopathies--kuru and transmissible dementia of the Creutzfeldt-Jakob type of man and scrapie and transmissible mink encephalopathy of animals; and, (2) fatal CNS diseases caused by conventional viruses associated with a protracted course, elevated proteins and CSF pleocytosis, histological evidence of inflammatory response and an immune response detectable in serum and CSF with titer ratios between CSF and serum frequently in excess of the ratios for other virus antibodies: defective measles in SSPE, new papovaviruses, including J.C., and SV40 from PML and BK virus from kidney transplant, rubella virus in congenital CNS infections, chronic encephalitis from herpes simplex and adenovirus type 32, and "old-dog" distemper in dogs, focal movement disorders in primates with RSSE virus and the certainty of measles and related viruses in some forms of multiple sclerosis.

The basic study of kuru has provided for the isolation and characterization of over two hundred virus strains from in vitro explanted tissues, primarily animal in origin: the development of a transformed human brain cell line associated with an RNA virus having a reverse transcriptase which is antigenically related to the Mason-Pfizer monkey mammary tumor virus; the development of a transformed cell line from explant cultures of a class II astrocytoma of the human brain; isolation of additional new simian foamy viruses and a clearer definition of the properties of these viruses as members of the group oncornaviruses. These studies have further led investigators in other laboratories to the successful isolation of the viruses of SSPE and PML.

Kuru: a heredofamilial disease of the Fore people of the Eastern Highlands of Papua New Guinea. In man the incubation period is estimated to be from 5 to more than 20 years following self inoculation during the practice of ritualistic cannibalism. The disease is transmissible to chimpanzees (incubation period 10 to 59 months), the gibbon ape (histopathological lesions in the absence of clinical disease), 5 species of New World monkeys (spider, 10 to 51 months; squirrel, 9 to 44 months; capuchin 10 to 45 months; woolly, 33 months; marmoset, 31 to 38 months; and two species of Old World monkeys, the rhesus with an incubation period of 8 1/2 years, and the mangabey. The virus passes through a Millipore filter of 450 nm and 220 nm, but thus far has been retained by filters of 100 nm and smaller size. The virus causes the disease in animals inoculated with brain tissue by the IC + IV routes, but may also be transmitted to animals inoculated by multiple peripheral routes (IP, IM, SC, IV), or by the IM route only. Human brain contains at least 10^6 infectious units per gram of tissue and in chimpanzee brain it exceeds 10^7 infectious units per gram. Virus is apparently widely distributed in visceral tissues of affected animals and disease has been induced in chimpanzees injected with pools of spleen, lymph node and kidney tissues. The virus is stable at 85°C for 30 minutes, can be lyophylized and stored at -20°C, and remains fully infectious when stored at -70°C in whole tissues or suspensions of tissues. The virus persists in vitro in explanted cultures of kuru affected chimpanzee brain maintained at 37°C for at least 70 days, but is not associated with CPE; it does, however, retain the capacity to induce fatal disease in primates following incubation periods not appreciably different from that in animals inoculated with suspensions of infected tissues. To date we have not been able to elicit a specific, direct or indirect antigen-antibody reaction employing specimens from naturally affected humans or experimentally infected animals, nor has it yet been possible to experimentally induce detectable levels of antibody in animals hyperimmunized with multiple doses of highly infectious tissues. Recently we have partially purified the virus by zonal density gradient and the fractions derived are being assayed for biological activity and biological, chemical, and physical properties of the virus. A number of attempts to assay the effectiveness of chemotherapy immunosuppressive therapy and other physical and chemical means of altering or delaying the disease in experimental animals have been tried without success. Of significance has been the study on the sequential development of histopathological lesions in the central nervous system of spider monkeys inoculated with kuru infected human brain tissue and sacrificed at specified intervals after injection. Preliminary data show that in as early as 3 to 4 months after IC inoculation, neuropathological lesions of status spongiosis and some gliosis can be detected by light and electron microscopic examinations of brain tissues from perfused animals. These studies are, for the first time, providing information on the pathways of progressive cellular alteration and destruction, neurophysiological alteration and imbalance at the membrane level all unduced by virus-cell interactions.

Transmissible dementia of the Creutzfeldt-Jakob type: The successful transmission of kuru subsequently led to the discovery that the Creutzfeldt-Jakob type of presenile dementia is also transmissible to the chimpanzee after asymptomatic incubation periods that have ranged from 11 to 26 months. The

disease can also be transmitted to 5 species of new world monkeys (spider, 21-37 months; squirrel, 13-25 months; capuchin, 29-39 months; woolly, 21 months; marmoset, 43 months); in addition, the disease has also been transmitted to 5 species of old world monkeys [bushbaby, 16 months; mangabey, 41 months; African green, 33-47 months; rhesus, 60-67 months; and the pigtail (histopathological lesions 2 months after inoculation)]. As previously reported, C-J disease is transmissible to domestic cats with an incubation period of 30 months; the virus has now been shown to serially be transmissible in cats and is associated with a dramatic reduction in incubation period of over 19 months to 11 months. Further, it appears that the incubation period is dose dependent since 10^1 and 10^2 dilutions have incubation periods of 20 and 22 months, respectively. The disease, like kuru, can be induced in animals inoculated peripherally only (IP, SC, IM) and although the virus is serially transmissible there has been no appreciable reduction in the incubation period. The virus passes through 450 nm and 220 nm Millipore filters but is held back by 100 nm and smaller filters. The virus persists and remains fully infectious for animals in explant cultures of human brain maintained in vitro in third serial subculture for over 255 days.

Isolations have been made from more than 30 human cases. Most cases have been sporadic in occurrence; however, we have recently isolated the virus from 2 well-documented cases of the familial type, indicating that this apparently genetically determined form of the disease is transmissible. In this context, familial cases of Alzheimer's disease appear to be more common. However, in some of these families the medical history of some of the individuals suggest C-J disease rather than Alzheimer's disease. From one such family we have recently transmitted the disease and the resulting pathology, restricted to the gray matter of the brain, is that of spongiform encephalopathy. It is significant to note that in the 10 year history of that phase of the program dealing with the isolation and characterization of viruses from human and animal CNS diseases there has been no evidence of animal to animal spread of the diseases by contact or other exogenous means within the more than 3,000 primates and over 30,000 small animals used to date.

An extensive review examines the 32 patients to date whose disease has been transmitted to a total of 60 animals and compares them with 71 patients whose disease has not so far transmitted. The two patient groups are not distinguishable from each other by the clinical and pathologic criteria examined.

It is significant to note, experimental SSE has been transmitted from the brains of 2 patients not suspected of having CJD during life: one had Degos' disease with evidence of CJD post-mortem while the other had familial Alzheimer's disease.

The laboratory continues to collect tissues (frozen and in tissue culture medium) from patients with a variety of neurologic disorders for inoculation and explant culture studies. Brain specimens collected in the past year include 26 from patients with ill-defined dementia, 15 with sporadic CJD, 1 familial CJD, 8 sporadic and 1 familial Alzheimer's disease, and 18 from other patients with 12 different diagnoses.

A specimen of particular interest is a brain slice in formalin from the recipient of a cornea transplant, probably the first human-to-human CJD transmission. A cornea transplant from capuchin monkey to capuchin monkey has been done in an attempt to duplicate this.

CJD brain in bulk has been purified by filtration and zonal centrifugation in collaboration with Dr. Siakotos and Dr. Bucana. Selected fractions are being inoculated into monkeys to assay for infectivity and studied by electron microscopy to determine the morphological characteristics of the purified preparations.

Other results include the transmission of CJD from human liver from 2 cases and also from a visceral pool. To date, experimental subacute spongiform encephalopathy has been transmitted by inoculation of tissue from 32 patients. More than 90 animals have come down with the disease on either primary or serial passage.

Thus, research continues in 3 major directions: (1) determining what is characteristic of those CJD patients whose disease is transmissible to primates as compared with those patients whose disease is not transmissible; (2) determining whether experimental spongiform encephalopathy can be produced in primates by inoculation of tissue from patients with disease other than CJD; and (3) further defining the physicochemical properties of the CJ agent.

Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus: (D.M. Asher, M.D., D.C. Gajdusek, M.D., H. Amyx, D.V.M., C.J. Gibbs, Jr., Ph.D.) As described in previous reports we are attempting to define the mechanisms by which intracerebral inoculation with some strains of tick-borne encephalitis virus produces chronic movement disorders in rhesus monkeys, as a model for our studies of chronic encephalitis with focal epilepsy in humans.

To date nineteen monkeys have been inoculated intracerebrally with the Vasil'chenko strain of tick-borne encephalitis virus from one to two and a half years ago. Five developed writhing choreoathetotic tremors of one hand and forearm, one with involvement of the toes of one foot as well. Another developed major motor seizures. Two animals with choreoathetotic movements, one normal animal without sequela after uneventful recovery from mild acute encephalitis, one with acute encephalitis and a control monkey inoculated with normal mouse brain were sacrificed by perfusion. Brain, CSF and kidney from seven monkeys (one of which had recovered from choreoathetosis about a year after onset) were examined for the presence of virus by serial passages in suckling mice; no virus has been isolated. Tissue cultures have been established from many organs including several areas of brain. Antibody responses to tick-borne encephalitis virus did not appear to differ between monkeys with and without choreoathetosis.

All monkeys with focal movement disorders, that were not sacrificed, reverted to normal by about 12 months post-inoculation. All monkeys which showed such disorders have been sacrificed from one to 2-1/2 years post-infection and

primary tissue cultures established from various areas of brain and from visceral organs. Virus isolation attempts by three serial intracerebral passages in suckling mice from these brains have been consistently negative.

Large paraffin sections from two perfused brains have recently been prepared. Preliminary studies are being conducted by Dr. Neal Nathanson at Johns Hopkins University.

An attempt to develop a satisfactory fluorescent antibody system was unsuccessful but these studies are being continued since it is felt that viral antigen is present in the brains of these animals.

Tissue from human patients with chronic focal epilepsy, spastic hemiparesis and dementia, and with a rapidly progressive fatal course, have been received, explanted successfully, and passaged into mice and tissue cultures; no agents have as yet been recovered. Attempts are being made to activate latent viruses in these cultures. Patients sera are being examined for antibodies to known arboviruses.

Tissue and cell culture studies: (N. Luber, L. Gilbert, M. Lewis, D. Asher, C. Gibbs, Jr., D.C. Gajdusek) Major emphasis has continued to be placed on the development and utilization of tissue and cell culture techniques in the study of slow, latent and temperate viruses. Such studies designed to isolate, identify and characterize viruses from human and animal tissues obtained at surgical biopsy and early autopsy from patients with chronic subacute degenerative diseases naturally acquired or experimentally induced have been most rewarding. During the period covered by this report additional data has been collected on the persistence of the viruses of kuru and C-J disease in in vitro explant cultures of brain tissue from naturally affected humans and experimentally infected animals; further, we have recently demonstrated that these viruses can, in the absence of observable CPE, be serially passed from infected explant cultures to BSC-1, Flow 3000 and HEK cell lines. These experiments suggest, for the first time, in vitro replication.

During the period covered by this report the following isolations have been made from non-human primate tissues in vitro: (A) foamy viruses--spider monkeys (6); capuchin monkeys (14); squirrel monkeys (5); rhesus monkeys (7); chimpanzees (30); mangabey (2); pigtail macque (1). (B) herpes-like viruses--spider monkeys (7); capuchin monkeys (7); squirrel monkeys (5); African green monkeys (1). Many of these isolates have been typed and data are reported in other sub-sections of this report; still others remain, as yet, unidentified.

Cell lines have been established on a variety of tissues obtained at necropsy from the following animals: chimpanzees (12); capuchins (5); squirrel (7); spider (4); African green (3); mangabey (1). The majority of these animals were killed in advanced clinical stage of kuru or C-J disease; however, cell cultures have been established with tissues from animals dying with transmissible papulosis atrophicans of Degos and familial Alzheimer's disease.

During the period covered by this report 77 specimens of viable tissues from 53 patients have been received into the laboratory. Twenty-three patients were diagnosed as having C-J disease while the remaining tissues were from a total of 30 patients with the following diseases: chronic encephalomyelitis (1), myoclonic epilepsy (2), Huntington's chorea (2), amyotrophic lateral sclerosis (1), Bruton's agammaglobulinemia with subacute dementia (1), subacute encephalitis (2), cranio-somatic ophthalmoplegia (1), PML (1), Degos syndrome (1), subacute cerebellar degeneration (1), diffuse cortical atrophy of unknown etiology (1), familial cerebral degeneration, several cases of leukemia, soft tissue sarcoma, metastatic renal carcinoma and one case of possible acute multiple sclerosis. Of the 23 C-J specimens, 8 failed to grow during the 60 days they were under study. Growth was obtained on all other tissues and cell lines established and seed aliquots frozen in LN. Viruses have not yet been isolated from any of the above cases. In one case given a provisional diagnosis of PML, toxoplasma were cultivated in vitro. Attempts to recover virus by fusion and co-cultivation with permissive cell lines as well as attempts to activate or "switch-on" latent and defective viruses by treatment of cultures with IUDR, BUDR, DMSO, temperature variation, were negative. In a single instance, a brain tumor, classified as a class II astrocytoma was submitted for study; shortly after explantations CPE was noted which consisted of a rounding and ballooning of small foci of cells. This has persisted through 19 serial sub-cultures. The CPE is herpesvirus-like and can be induced by sub-inoculation into human foreskin, human embryo kidney, rabbit kidney, human fetal brain, and African green monkey kidney. The virus isolate is being characterized.

In addition to the studies on human tissues, this laboratory has been heavily involved in in vitro studies on tissues from sub-human primates inoculated with human tissues and viral isolates from these tissues. As previously reported, and earlier noted in this report, more than 200 strains of viruses have been isolated from animal tissues. During the past year we have established sub-human primate cell lines from the following disease categories: C-J disease--chimpanzees (3), spiders (3), cynomolgus (2), squirrels (3), capuchins (1) and rhesus (3); kuru--chimpanzees (3), capuchins (1); scrapie--squirrels (2). These lines included not only CNS tissues but visceral tissues as well. In addition to determining "marker systems" for the presence of the viruses causing the related diseases these cell lines are being investigated in collaboration with Dr. Todaro, NCI, and Dr. Kohne, Scripps Institute, for type "C" particles, transformation, and reverse transcriptase. From 8 of the animals additional strains of foamy viruses have been isolated. In collaboration with NCI investigators we are conducting studies on cell lines chronically infected with WM1504 virus, associated with naturally occurring lower motor neuron disease in mice and JC8CV1 hybrid Creutzfeldt-Jakob and CV-1 transforming agent. Finally, cell lines have been established from the following normal animals: ferret (brain), gorilla (brain, cerebellum, spinal cord), chimpanzee (brain).

Simian foamy viruses: (D.C. Gajdusek, C.J. Gibbs, Jr., P. Brown, T. Tsai, J. Hooks, M. Sulima, H. Amyx, A. Bacote, M. Lewis) In the past two years in this laboratory 2 new foamy viruses of chimpanzees, Pan 1 and Pan 2 (or Foamy

viruses 6 and 7), were isolated and described in detail, and an additional foamy virus, designated 8, was isolated from a spider monkey and also fully described. These viruses belong to a group of RNA, heat and chloroform sensitive, medium-sized viruses containing RNA-dependent DNA polymerase, an enzyme exclusively associated with transforming, and in most cases, tumor-producing viruses.

Two of the as yet incompletely explored problems with these viruses are their behaviour in the individual host (pathogenesis) and in host populations (epidemiology). Until now it has been nearly impossible to make a satisfactory complement-fixing antigen to any of these viruses, and all serological studies have been done using the neutralization test. This test is time-consuming on the one hand, and on the other hand measures a long-lasting antibody so that recent, as opposed to distant, infection cannot be distinguished. Furthermore, from the point of view of virus classification, the neutralization test is the most discriminating of immunological indices, and has indicated almost no cross-reactivity among the 8 foamy viruses thus far isolated. Complement-fixation testing, however, might reveal common group relationships impossible to determine by other means.

For these reasons, an intensive effort has been made to produce a complement-fixing antigen to one of the foamy viruses isolated in this laboratory, Pan 1 virus. Large volumes of virus-infected tissue culture cells were grown over a several month period, and the cells and supernatant fluids frozen and thawed, ultracentrifuged, the pellets resuspended in 1/25th the original volume, and the suspension sonicated. This preparation was found to have excellent complement fixation activity against hyperimmune Pan 1 antisera.

Initial studies are underway to correlate results of neutralization tests with complement-fixation tests in the sera of chimpanzees having neutralizing antibody to either Pan 1 or Pan 2 virus, to both viruses, or to neither virus. Serological screening is also being undertaken on a large number of chimpanzees in captivity, and in their wild habitat, on other primate and non-primate animal species, and on humans. Preliminary results indicate that the complement-fixing antigen of Pan 1 virus also reacts with antibody to Pan 2 virus, and may even extend to the foamy viruses found in monkeys. No human yet tested has complement-fixing antibody to the Pan 1 antigen. Virtually all of the chimpanzees tested have antibody, suggesting that the virus is behaving as a chronic latent infection in this host, with continual low level stimulation of immunologically competent cells. One chimpanzee has been found to have converted from a negative to a positive reaction during his first year in captivity in this country. Studies of pathogenesis of the infection will be set in motion shortly.

Studies on transformed human brain tissue in vitro and characterization of associated virus: (C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D., Monica Lewis, S. Chou, M.D., G. Todaro, M.D., S. Aaronson, M.D., W. Parks, Ph.D.) In previous reports we have described the in vitro transformation of human brain cell cultures, derived from a patient with Creutzfeldt-Jakob disease. Virus particles morphologically akin to oncogenic RNA viruses are

When newborn hamsters were observed for 100 days after SC inoculation, tumors began to appear and by 230 days 54% of animals (none of controls) had tumors which enlarged progressively without evidence of either metastasis or remission. The tumors were multiple encapsulated masses interpreted by a consulting pathologist as undifferentiated carcinomas. Tissue cultures were readily established from these tumors and were oncogenic for 100% of newborn hamsters within 40 days of inoculation.

Serological identification of chimpanzee adenovirus strain A-226-1: The agent was identified serologically as an adenovirus in the CF test using commercial adenovirus grouping serum and control human adenovirus antigen and uninfected human diploid cell control antigen. A rabbit serum prepared against A-226-1 virus had an adequate neutralizing antibody titer, but unfortunately was highly anticomplementary.

We attempted to identify the virus further by neutralization test using 100 $TCID_{50}$ of virus with 1:10 dilutions of type specific antisera to the known human adenoviruses 1-31 as well as to two newer human adenoviruses 32 and 33, and against 22 monkey adenoviruses which were weak or nonagglutinators of rat erythrocytes. We also performed neutralization tests using rabbit antisera against five chimpanzee adenoviruses previously isolated from mesenteric lymph nodes of chimpanzees in our colony, as well as typing sera for two chimpanzee adenoviruses described by others. None of the human or simian typing sera neutralized our virus. Antisera for several other strains of chimpanzee adenovirus, described in the literature, including one which is a nonhemagglutinator, could not be obtained.

Biology of infection with chimpanzee adenovirus: Virus was isolated from the urine of chimpanzee A-226 on each of five occasions; the last isolation was made more than a year after the virus was first detected. More than 10^5 $TCID_{50}$ of the 11-month isolate was readily neutralized by a 1:10 dilution of antiserum to the first isolate, demonstrating that it is the same virus persisting in the urine. Urine from 15 other chimpanzees have been examined. Urine from 7 animals yielded isolates which produce similar CPE in tissue cultures, one of these chimpanzees on two occasions. Isolates from two animals were neutralized by antiserum to A-226-1 virus. One other isolate was apparently not neutralized; this finding remains to be repeated and if confirmed the isolate will be identified. Isolates from four animals have not yet been tested satisfactorily. Two of the animals yielding isolates were tested within a week of their arrival in our colony.

The urines were all bacteriologically sterile and all save one had unremarkable sediments. One animal which arrived in the colony appearing ill was found to have about 100 white cells per high power field of centrifuged urine; it was treated empirically with antibiotics and improved; subsequent urine sediment was normal. No adenovirus has been isolated from kidneys of any chimpanzees autopsied by our laboratory, nor have any pathological changes been noted in kidneys at autopsy. Stool and throat specimens were obtained from chimpanzee A-226 at the same time the urine contained virus; the stool yielded nothing and the throat a foamy virus. A-226 has previously been demonstrated to have simian foamy virus type 7 in her leukocytes.

Sera of 60 chimpanzees tested by neutralization test at dilutions of 1:8 or less were found to neutralize 100 $TCID_{50}$ of A-226-1 virus. Serum from chimpanzee A-226 herself neutralized virus at a dilution of at least 1:1024. Sera of four chimpanzees recently acquired by our colony were obtained on the day of arrival prior to contact with other animals; all four neutralized virus at a dilution of 1:8. Sera obtained from eight chimpanzees shortly after their arrival at various times between 1963 and 1968 were paired with sera from the same animals in January 1974. All 8 chimpanzees had serum antibodies in 1974 and 7 of the 8 also had antibodies in the earlier sera, one as early as 1963. One animal had no antibodies demonstrated in a serum collected in April 1966.

Sera of 22 past and present workers in our laboratory were screened at 1:5 dilution against 100 $TCID_{50}$ of A-226-1 virus. Nineteen had no neutralizing antibodies. Three animal caretakers had antibodies at dilutions of 1:64 to > 256. Two of these people were found to have no antibodies in sera obtained relatively early in their terms of employment in 1964 and 1968 respectively. The third had no serum available prior to 1965, two years after he began to work with chimpanzees, at which time he already had serum antibody. Urine from the one worker still in the laboratory was cultured; no virus was isolated.

A preliminary survey of 39 monkeys (8 species) from our colony using sera at 1:5 dilution and 10 $TCID_{50}$ of virus demonstrated that high percentage gave apparent neutralization; however, a subsequent screening using sera diluted 1:8 and 100 $TCID_{50}$ failed to confirm this and the question of specific immunity among monkeys remains in doubt.

Persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys: (D. Asher, C.J. Gibbs, Jr., D.C. Gajdusek, D. Lang, H. Amyx, M. Sulima, A. Bacote) Chronic asymptomatic viruria of monkeys with cytomegalovirus is similar to that of man and simian infections may be useful models of human disease.

A group of healthy young rhesus monkeys were examined in 1968 and again in 1972. Most monkeys had cytomegalovirus in the urine at both times. Rhesus cytomegalovirus has not previously been isolated; although similar to cytomegalovirus of the African green monkey in biological properties the rhesus virus was not neutralized by sera which neutralize the African green virus, nor by antisera to human cytomegalovirus. The complement fixation test showed that rhesus, African green and human cytomegaloviruses shared some antigenic determinants. When a rhesus monkey chronically infected with rhesus cytomegalovirus was inoculated with human cytomegalovirus no neutralizing antibody to the human virus appeared, but there appeared to be an increase in neutralizing antibody to the homologous rhesus virus.

Rhesus monkeys in our colony continue to shed cytomegalovirus in the urine six years after this phenomenon was first detected. After several unsuccessful attempts to demonstrate cell-mediated immunity to CMV in these monkeys a concentrated antigen was prepared. With this antigen lymphocyte transformation

present in the transformed cells. Our objective has been to further characterize the isolated virus, attempt to transmit C-J disease with both 10% suspension of the human brain and the transformed cell line, and finally to determine the relationship of this virus with human disease.

The virus particles present in the human brain cell cultures do not fit the morphological definitions of either type C or type B particles; they do resemble Mason-Pfizer monkey virus (MP-MV) described by Chopra and Mason. In collaborative studies, we have transmitted the S.G. virus to a human sarcoma cell line and demonstrated in these cells an RNA-dependent DNA polymerase that immunologically cross-reacts with the DNA polymerase of MP-MV. The S.G. transformed cell line does not contain reverse transcriptase activity. Also, the protein patterns of the S.G. virus and MP-MV, as analyzed by polyacrylamide gel electrophoresis, are similar in the number and relative size of peaks. Further studies are required to determine if the S.G. virus and MP-MV are separate isolates of a similar virus. Recently a virus similar to both the S.G. and MP-MV viruses was isolated from humans in the U.S.S.R., thus confirming the validity of the relationship of S.G. to the human patient.

Isolation and characterization of CMV from urine of chimpanzees: (D. Asher, H. Amyx) A herpes virus (cytomegalo-type) cytopathic effect was induced in MA-184 cell lines inoculated with urine from chimpanzees A-173 and A-226. Urine from one additional chimpanzee (A-217) has been inoculated into HEK and MA-184 cell lines.

Isolation and characterization of adenovirus from the urine of chimpanzees: (D. Asher, D.C. Gajdusek, C.J. Gibbs, Jr., J. Hooks, H. Amyx, N. Luber, H. Barki, M. Sulima, A. Bacote) We isolated from chimpanzee urines agents which produced a cytomegalo-like cytopathic effect in W138 cultures, One of the two earliest isolates, designated A-226-1, was present in relatively high titer, > 10^4 $TCID_{50}$ per ml urine, produced CPE within ten days and readily attained adequate concentration when passed twice in W138 cultures (about 10^7 $TCID_{50}$ per ml of cells plus fluids); it was therefore selected for further study.

Properties of A-226-1 isolate: The virus was found to be relatively sensitive to FUDR, resistant to chloroform, and by electron microscopy had nuclear particles of 600 to 900A with dense coves typical of an adenovirus. No particles resembling adeno-associated virus were seen. The virus produced small yellow to green intranuclear inclusions with no cytoplasmic inclusions by acridine orange stain. CPE was produced in human embryo kidney as well as in W128 and MA-184 human diploid fibroblasts and in primary cultures of chimpanzees, rhesus and African green monkey cells, but not in kidney cells of owl or squirrel monkey or of rabbit. No signs of acute illness appeared in newborn mice or hamsters after IC or SC inoculation. Suspensions of virus containing > 10^5 $TCID_{50}$ per ml failed to agglutinate guinea pig, rhesus or rat erythrocytes at either 4° or 37° C and addition of antiserum to human adenovirus 5 which has been reported to increase agglutination of some low numbered heterotypic human adenoviruses did not change the results.

was demonstrated. Additional attempts to improve antigens are under way. When better reagents for rhesus and other simian CMVs and human CMV have been obtained, the response of monkeys to heterologous immunizations will be studied.

The antigenic relationship of rhesus cytomegalovirus to the newly-isolated chimpanzee cytomegalovirus will be studied. Humoral and cellular responses of rhesus monkeys chronically infected with rhesus cytomegalovirus to inoculation with homologous and heterologous cytomegaloviruses will be examined.

Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates: (M. Lewis, S. Chou, M.D., E. Cutching, Ph.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) In the course of routine tissue culture work in our laboratory, we have observed herpes-like CPE in several of our simian cultures. Upon inoculation into stationary tube cultures of MA-184 these isolates produce characteristic herpes-like CPE. Thus far, isolations have been made from the following: capuchins--AP-18 brain cortex; AL-5 spleen and lymph node; AL-14 spleen; AL-3 spleen; AL-37 spleen and skin. Spider monkeys--S-232 brain, lymph node and spleen. Squirrel monkeys--O-142 spleen and kidney; O-157 spleen and kidney; O-39 spleen. African green monkey--680Z kidney. To date, however, only two of these agents have been characterized, i.e. AP-18 cortex and AL-5 spleen isolates.

In fibroblastic cells (human skin and muscle, human foreskin, W138, Flow 3000, and squirrel monkey fetal lung) the isolate induces a typical herpes virus cytopathic effect (CPE) consisting of rounded and ballooned cells. The capuchin herpes-like virus does not induce CPE in human embryonic kidney, owl monkey kidney or squirrel monkey kidney cells. By electron microscopy, the virion is observed both in the nucleus and cytoplasm and morphologically resembles a herpes virus. Acridine orange staining of AP-18 infected W138 cell cultures revealed small DNA containing nuclear inclusion bodies. The virus is chloroform sensitive, is inactivated at 56° C for 30 minutes, is extremely sensitive to freezing and thawing and lyophilization, and passes through a 220 nm but not through a 100 nm Millipore filter. Hemagglutination and hemadsorption studies were negative for a variety of erythrocytes. The isolate appears to be deficient in thymidine kinase, since it is not inhibited by BUDR (40 mg/ml) but is inhibited by FUDR (10^2 M concentration). The herpes-like virus did not induce clinical disease or abnormalities in newborn mice, embryonating hen's eggs and a capuchin monkey.

Neutralizing antibody was present in the serum of the capuchin monkey from whom the isolation was made. The virus is not neutralized by 15 different prototype herpes-virus antisera nor has it been possible to demonstrate any neutralizing antibody in the sera of guinea pigs, and a capuchin monkey hyperimmunized with the agent. Neutralizing antibody to the isolate was demonstrated in 7 of 16 capuchin monkey sera tested whereas no neutralizing antibody was detected in the serum of 16 humans, 6 rhesus monkeys, 5 squirrel monkeys, 10 spider monkeys and 15 chimpanzees tested. During this year we

were successful in preparing immune serum in rabbits hyperimmunized with AP-18 virus. This sera was tagged with FITC and an FA test developed which shows a one-way cross between AP-18 and AL-5 viruses.

There has been a renewed interest in herpes viruses due to the implication of 2 simian herpes viruses, H. Saimiri and H. Ateles, in oncogenesis in primates.

Studies on the characterization and nature of the viruses of scrapie, transmissible mink encephalopathy and visna diseases: (C.J. Gibbs, Jr., Ph.D. D.C. Gajdusek, M.D., M.P. Sulima, A.E. Bacote, H. Amyx, D.V.M., Roger Traub, M.D., Eric French, Ph.D., A. Siakotus, R. Latarjet, L. Hertzberg, M.D., M. Dubois-Daleq) As a disease entity, scrapie manifests clinical signs and neuropathological lesions remarkedly similar to those observed in human patients with kuru and Creutzfeldt-Jakob disease. Scrapie is caused by a serially transmissible, atypical virus which has a size of approximately 20 to 30 nm. In the non-purified state the virus is unusually resistant to inactivation by ultraviolet light, formalin, heat, beta-propriolactone, but is sensitive to the action of phenol and diethyl ether. In cesium chloride it has a buoyant density of about 1.32. The virus induces a subacute progressive degenerative disease of the nervous system in sheep and goats naturally infected and produces the same fatal disease when injected in small amounts into mice, rats, hamsters, gerbils, voles, and mink. Of significance is our recent report on the transmission of scrapie to the cynomolgus monkey after an asymptomatic incubation period of five years and five months, the first successful transmission of this virus to a subhuman primate; additional transmissions of several strains including sheep, goat and mouse origin of scrapie have now been accomplished in squirrel monkeys (incubation period 14 months). The squirrel monkey thus becomes the first experimental host susceptible to each of the prototype spongiform encephalopathies. This further suggests the possibility that the original hosts and perhaps even the reservoirs of kuru and Creutzfeldt-Jakob disease may be animals. Ultracentrifuge zonal gradient studies indicate maximum peaks of infectivity associated with peak zones of protein and nucleic acid in brain tissue suspensions; these data strongly suggest that scrapie is associated with small fragments of nucleic acid (probably double stranded RNA) that is tightly bound to plasma membrane. Using the technique of Diener, we were not able to isolate small molecules of naked nucleic acid from scrapie infected mouse brain and spleen due to technical inactivation of the virus by phenol extraction. Continued attempts to demonstrate specific antigen-antibody reactions to scrapie remain unsuccessful. The immune mechanism of the scrapie infected host remains functional and without alteration on both the humoral and cellular levels. Administration of large doses of interferon, poly I-poly C, x-irradiation and antilymphocytic serum have all failed to interrupt or alter the onset or course of this fatal disease of animals. An extensive electron microscopic search for virus in the fractions of brain suspension obtained by density gradient banding the agent in zonal ultracentrifuge have revealed maximum infectivity is present in those fractions which are composed almost entirely of plasma membrane. Although we can see no consistent evidence of fully formed mature virions, we are certain that degenerative processes seen must represent the effect of virus production and maturation,

e.g. alteration of the plasma membranes of cells resulting in fragmentation of the unit membrane and the filling of intraneuronal vacuoles with this membraneous material; in vivo fusion of astrocytes with astrocytes and astrocytes with neurons, further suggesting alteration of the membrane.

With the arrival of Drs. French and Hertzberg, visiting scientists from Australia and Scotland, respectively, we intensified studies on scrapie virus to include: (1) Attempts to grow scrapie virus in tissue cultures of a range of mouse and sheep tissues and to obtain, in large quantities, cells chronically infected with scrapie virus; (2) attempt to detect cell mediated immune mechanisms in animals infected with scrapie; (3) attempt to infect mice, hamsters and guinea pigs with kuru and Creutzfeldt-Jakob disease (CJD) brain specimens; and (4) attempt to shorten the incubation period of scrapie in mice.

As a preliminary to further work, a stock of scrapie virus was prepared from mice in the advanced stages of clinical scrapie following inoculation with scrapie SPG9 M10-11 and zonal gradient experimental fractions 23/26. Histological examination of the portions of the brains of these mice revealed extensive spongiform changes typical of scrapie infection. A 10% suspension of the affected mouse brain was prepared in phosphate buffered saline (pH 7.4) by grinding the brain in a frozen mortar, centrifuging the resultant suspension at 2500 rpm for 60 minutes. Aliquots of 0.2 ml of the supernatant material was stored in ampoules at -85° C. Subsequently, a stock of normal aged mouse brain suspension (NMBS) was prepared and stored in a similar way.

Growth of scrapie virus in tissue culture. Tissue cultures of suckling mouse (NIH strain), kidney, spleen and thymus cells were readily initiated by the explant technique on several occasions. As a control on scrapie-affected mouse tissue cultures, spleen and kidney tissue from normal aged (older than 12 months) mice have been established and some of this material has been stored in liquid nitrogen. The tissue cultures have been grown in 20% fetal calf serum in Eagles No. 2 medium and maintained in the later stages in maintenance medium consisting of 5% fetal calf serum in Eagles No. 2 medium. The medium has been changed on all cultures twice a week.

Sheep tissues have also been obtained from a 1-2 year old normal ewe being sacrificed at Johns Hopkins Hospital for lymphoid tissue (spleen and kidney only was obtained) by arrangement with Drs. R. Johnson, Narayan and Silverstein, who intimated that the donor animal was from their flock of normal sheep and had not been in contact with any known diseases of sheep; (b) from a lamb less than one week old which was one of twins born to a normal ewe in a flock of normal sheep maintained at Fort Detrick. The other twin died soon after birth from an undetermined cause. Cell cultures were prepared with kidney, adrenal, thyroid, thymus and brain.

Inoculation of tissue cultures with SMBV. Cultures of the various tissues in low passage level (1-4) from the original explants were inoculated with a 1/50 to a 1/200 dilution of stock scrapie virus in growth medium and 3 ml of this virus suspension was added to each falcon flask (30 ml) of

tissue. The virus was left in contact with the cells for 4 to 5 days before being removed and replaced with fresh growth or maintenance medium. Strategic supernatant fluids from each culture were harvested and stored at -85° C for subsequent titrations in mice.

The controls were uninoculated cultures, but later these consisted of the cultures that had been inoculated with a 1/50 to 1/100 dilution of normal mouse brain suspension. The cultures were inspected regularly and photographed from time to time to record changes occurring in both inoculated and uninoculated cultures. Selected cultures were examined by electron microscopy. The following tissues were used in these experiments: (a) mouse kidney; (b) mouse spleen; (c) mouse thymus; (d) sheep brain; (e) sheep spleen (adult only); (f) sheep kidney; (g) sheep adrenal; and (h) sheep thyroid.

No evidence of gross cytopathology in any of the inoculated mouse tissue cultures was obtained. The changes that were noted consisted of some aberrations in the nucleus of some mouse thymus cells and evidence of cell lysis in some cultures after 40 days incubation with aging degeneration appearing in both control and scrapie infected cells at about 50 days. In some kidney cultures, clones of fibroblastic cells appeared around 50 days and began to overrun the other cells in the culture.

The results with the sheep tissue cultures will be considered in relationship to the origin of the sheep tissues. (a) Adult sheep tissues obtained from Johns Hopkins Hospital, Wilner Eye Institute: Tissue cultures of spleen and kidney cells at the third passage level were inoculated with scrapie virus; controls were uninoculated cultures. The kidney cell cultures showed no evidence of cytopathic effect in either the scrapie inoculated or control flasks until about 30 days when aging degeneration appeared in both control and inoculated cultures. The sheep spleen cultures were more interesting. Some double and triple nucleated cells were noted in these cultures before inoculation. Following inoculation these increased in frequency in both the inoculated and the control series. Electron micrography of both series at 16 days post-inoculation revealed some cells in both series with budding virion-like structures from the cell membrane and having the general morphology of visna virus (see separate section of this report for description of attempts to identify this agent). (b) Neonatal lamb tissues obtained from Fort Detrick. Tissues at the first or second passage level were inoculated with SMBV; the controls were uninoculated cultures from duplicate flasks for each tissue. The following tissues from this lamb: thymus, adrenal, thyroid and brain, were used. In both control and scrapie inoculated cultures of thymus, adrenal and thyroid cells focal areas of CPE appeared at 6 to 7 days post-inoculation. These increased to envelop almost the entire culture and then new cells began to appear in the spaces left by the contracting dead cells, and by about 35 days had almost covered the previously devastated areas. No signs of CPE appeared, either in the inoculated or control cultures of brain cells, but at about 30 days the cell sheets in most of the cultures contracted and floated off the flask. New cells regenerated slowly in both control and scrapie-inoculated cultures. Sheep kidney cells showed no evidence of specific CPE until general aging degeneration appeared in both the scrapie infected and

control cultures at about 30 days. Supernatant fluid from all these cultures has been collected and stored for titration for infectivity in mice.

Growth of tissues from mice infected with scrapie. Mice dying of scrapie, and from the same lot that provided the stock scrapie mouse brain suspension, were sacrificed and attempts were made to grow their tissues by explanting. Cultures of kidney cells from these mice were established slowly and after 45 days only one flask had a reasonable sheet of cells. This culture was trypsinized, but failed to really ever again establish a good cell sheet and, finally, this culture was terminated 23 days after trypsinization. The possibility that following trypsinization there was a massive release of virus that killed the cells, has to be considered and should eventually show itself in an increased titer in the supernatant medium harvested after this time. Spleen from scrapie-infected mice fairly readily established cell sheets in 7 out of 8 flasks within 45 days, but by day 75 three of these 7 had degenerated and had to be abandoned. These cultures that survived were eventually trypsinized and established as viable cultures. The cells from one of these flasks is in its fourth passage and the other in its second passage. An aliquot of these cells will be stored in liquid nitrogen and the other cultures maintained for as long as possible with periodic passages and collections of culture medium for titration in mice for viable scrapie virus.

One culture was examined by electron microscopy by Dr. Dubois-Daleq who observed some cytoplasmic inclusions of curled membranes in both these and control tissue cultures of mouse spleen cells.

Brain tissue from the scrapie infected mice could not be established in tissue cultures either by explanting or trypsinizing the tissue. This is at variance with other workers' results and no reason for the failures on several occasions which we have experienced can be offered.

The visna-like agent detected in sheep spleen cell cultures is being investigated by attempting to grow it in the other cell cultures, e.g. sheep choroid plexus cells, HeLa cells, and other established cell lines. Replacement of the fetal calf serum in the maintenance medium by 5% horse serum (known to be free of inhibitors of visna virus) has resulted in an increase in the size and frequency of syncytia in tissue cultures of the spleen cells. Attempts are being made to (1) concentrate the agent for studies by electron microscopy; and (2) identify it by serological methods.

Attempts to detect cell mediated and other immune mechanisms in scrapie infected animals. It had been hoped to fairly readily obtain cultures of cells chronically infected with scrapie virus. This has proved a more difficult and uncertain task and the results of titrations for infectivity of the supernatant fluids from the various tissue cultures from scrapie infected mice and tissue cultures inoculated with scrapie agent will need to be obtained before much progress can be made with experiments on cell mediated immunity. However, if time had permitted, an investigation of the inhibition of migration of leukocytes from capillary tubes in the presence of semi-purified fractions of scrapie mouse brain would have been undertaken.

Similarly, establishment of chronically infected scrapie tissue cultures may have provided the opportunity to apply several techniques now being exploited successfully in other systems, e.g. blast transformation of lymphocytes and the release of radioactive chromium from such cells by suitably "armed" lymphocytes.

In collaboration with Dr. Andre Nahmias of the Department of Pediatrics at the Emory University School of Medicine, Atlanta, Georgia, five tissue culture systems were examined by a modified fluorescent antibody technique in an effort to detect specific antigens in cells affected with scrapie; and specific antibody in the serum collected from animals at various stages in the course of infection with scrapie virus. The results of these experiments showed no clear-cut evidence of any serological reaction between the cells and sera. The small degree of fluorescence observed in some cells with some sera cannot be interpreted until the results of mouse titrations for scrapie virus become available on these cultures.

Attempts to shorten the incubation period of scrapie in mice. The effect of pyronin Y on growth of scrapie virus. From work on the reactivation of ultraviolet irradiated E. coli and the effect of certain inhibitors of the exonucleases operating in this process, it was reasoned that if the scrapie agent is integrated into the nucleic acid of the cell and then excised out again as a defect by the exonuclease this might account for the long incubation period of scrapie. It was further argued that inhibition of the exonuclease might shorten the incubation period.

Pyronin Y, a dye having an affinity for nucleic acid, is known to have a powerful inhibitory effect on the exonucleases of E. coli. Experiments were carried out by incorporating small quantities (0.0001%) of pyronin Y into the culture medium used to nourish tissue cultures of mouse and sheep tissues infected with scrapie virus. At this concentration the dye had no deleterious effect on the cultured cells. Incorporation into cells was observed by electron microscopic studies and by a slatey-purplish lor of the cells when centrifuged into a button for subcultures. No difference was observed in the cell sheets of flasks with and without pyronin Y in the culture medium in the presence or absence of scrapie mouse brain material. Mice were offered drinking water with up to 0.1% pyronin Y in solution. They appeared to be unaffected by this treatment over a period of four months and mice have been maintained on 0.01% pyronin for in excess of five months without obvious clinical disease.

Recently it became known to the writer that while caffeine and pyronin are potent inhibitors of bacterial exonucleases, they are apparently without effect on mammalian cell exonucleases. The compound reductone is reported to inhibit mammalian cell exonucleases and experiments should be started to test this compound in tissue cultures and in mice for its ability to alter the course of scrapie infection in these host systems.

Research workers at the USDA laboratories in Denver, Colorado have observed and described an "auto-inoculation" technique which they claim shortens the

incubation period of bluetongue in sheep inoculated with cattle strains of bluetongue virus. The technique involves the removal of a small quantity of peripheral blood at a strategic time in the incubation period and the injection of this blood intramuscularly into the same sheep.

Experiments have been commenced in collaboration with Dr. Gerald Ward of the Public Health Research Institute of New York in Otisville, New York, to ascertain whether "auto-inoculation" of blood from mice infected with scrapie virus can shorten the incubation period of scrapie in mice.

Clinical manifestations of experimental kuru and Creutzfeldt-Jakob disease in the chimpanzee: (H. Amyx, D.V.M., D. Asher, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) Careful observations of clinical course, electroencephalograms, blood and spinal fluid findings allow better comparison of the experimental conditions with natural human diseases and correlations of clinical manifestations with histopathological changes.

Over forty chimpanzees with experimental kuru have been studied. All had signs of diffuse brain disease with the earliest and most severe evidence of degeneration of cerebral cortex followed by less prominent cerebellar and basal ganglia involvement. Signs of disease in medulla and spinal cord were less common and tended to appear only late in its course. There was no disturbance of function of peripheral nerves or of muscles nor of any primary disease outside the central nervous system. Electroencephalograms were diffusely abnormal. Spinal fluid proteins rose slightly but significantly late in disease. There were no consistent or significant primary changes in blood chemistries or hematologic parameters. The clinical picture of experimental kuru in the chimpanzee correlates well with histopathological findings and is generally similar to human disease from which it differs in having relatively more severe cortical and less severe cerebellar involvement. Experimental Creutzfeldt-Jakob disease in the chimpanzee resembles kuru but has a shorter incubation period, more rapid clinical course, and prominent myoclonic jerks, paresis of limbs and sometimes somnolence not found in experimental kuru.

Electroencephalographic, spinal fluid and blood studies of chimpanzees with experimental Creutzfeldt-Jakob disease are in progress.

Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections: (H. Amyx, D.V.M., D. Asher, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D., R. Traub, M.D., R. Garruto, Ph.D., E. French, Ph.D., L. Hertzberg, M.D., M. Sulima, A. Bacote, W. Greer, D.V.M.) The most significant factor affecting the NINDS animal colony was relocation from the Patuxent Wildlife Research Center to Building 567 at Fort Detrick. Some immediate problems were felt but an overall beneficial effect is now in evidence.

Approximately ten animals were lost from pneumonia or diarrhea which appeared to be a direct consequence of the stress of moving. One chimpanzee died of sequelae to a debilitating heat stroke episode from crate shipment. A similar

loss occurred amongst animals shipped to our contract facilities at the Gulf South Research Institute, New Iberia, Louisiana.

The first few months of cage confinement presented problems for those animals which were formerly gang caged. Many had arms caught in cages, fingers caught in feeders, etc. After a few months these incidents stopped altogether.

The stress of moving also had a significant effect on animals with clinical neurologic disease. Some appeared to progress more rapidly through the clinical course of disease (L-656-Kuru; 719-Z-CJD; O-106-Scrapie) while others died suddenly with no gross lesions at necropsy (S-236-CJD; Pax 34-Kuru). More importantly, animals which had previously been normal started to show clinical signs of neurologic disease (O-105,O-107-Scrapie; AP-21, AP-41-CJD; Cat #3, Cat #4-CJD). We have long suspected that stress might well precipitate the onset of clinical disease in inoculated animals. Studies have been initiated to determine if the incubation period can be significantly reduced.

The level of intercurrent infections in the animal colony appears now to be at its lowest level ever. However, serious infectious diseases are still present. Three isolations of Entameba histolytica, one of Balantidium coli, one of Shigella flexneri, and one of a Group B Salmonella have been made from diarrhea cases in the past several months. Only one of these (B. coli) was fatal.

Eleven baby monkeys have been born since the move to Fort Detrick and many females are pregnant at this time. The colony as a whole has adapted well to an indoor, caged environment.

Gorilla growth and development studies, virus patterns and blood group genetics in the great apes. Studies are in progress in cooperation with the Gulf South Research Institute to elucidate growth and motor development and endocrine patterns, as well as polymorphic blood groups among the great apes. Studies of early as well as late acquisition of virus antibody patterns in "normal" non-inoculated gorillas are also continuing. Such studies will further our knowledge of non-human primates which have been so extensively used in our research programs.

Studies on the polymorphonuclear depressing factor in multiple sclerosis and other slow infections of the nervous system: (P. Brown, M.D., D.C. Gajdusek, M.D.) Carp et al have reported that suspensions of brain tissue from human patients with multiple sclerosis and from mice with scrapie when injected into mice induces an alteration in the percent polymorphonuclear neutrophils in clinically normal mice after inoculation. These investigators further reported recovery of an agent that could be serially transmitted in mice, was self replicating and has a size of 25-50 nm. In an effort to confirm these findings a series of experiments were conducted in several lines of inbred and randomly bred mice inoculated with human derived MS brain, including the Carp inoculum, and mouse derived scrapie viruses. Complete blood counts including differential leukocyte counts were performed. We have been unable to confirm the finding of decreased percentage of

polymorphonuclear neutrophils in mouse peripheral blood after inoculation with materials from multiple sclerosis patients and materials from mice with scrapie.

Development of serological and immunological test system for use in the study of slow infections of the CNS: (D.C. Gajdusek, M.D., C.J. Gibbs, Jr., Ph.D., P. Brown, M.D., F. Cathala, M.D., R. Benfante) During the period covered by this report serum and CSF specimens from humans with kuru, C-J diseases, Alzheimer's disease, chronic encephalitis with myoclonus and focal epilepsy, and a wide variety of other subacute chronic CNS diseases as well as pre- and post-serum and CSF from animals with experimentally induced diseases have been tested by direct and indirect complement fixation, mixed hemagglutination, hemadsorption, direct and indirect FA, and, for antibody to lytic and non-lytic neoantigens at cell surface loci in cell lines derived from human and animal CNS tissues. To date no specific antigen-antibody systems for kuru, transmissible dementia of the C-J type, or scrapie have been developed.

Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remissions and exacerbations: (P. Brown, M.D., F. Cathala, M.D., C.J. Gibbs, Jr., Ph.D., D.C. Gajdusek, M.D.) Studies to establish infection as the etiology of some forms of multiple sclerosis have been continued and expanded during the past year. Recent findings in our laboratory have confirmed the earlier observation that a significant number of MS patients have antibody to measles virus in their CSF, in sharp contrast to non-MS control humans. A second group of serum and CSF specimens from additional humans with MS had antibody to vaccinia virus, as previously reported by Kempe et al in Colorado. Virus particles morphologically similar to myxovirus-paramyxovirus nucleocapsids have been seen by EM in plaque and periplaque areas of brain from humans with MS. ter Muelen et al, have reported the isolation of "cold-mutant" strain of para-influenza type 1 virus from 2 cases of MS. However, we have not been able to demonstrate significant levels of antibody to para-influenza 1 virus in our collection of serum/CSF specimens from MS patients. Our data does, however, support our earlier hypothesis that some types of MS have a viral etiology and, in at least some instances, a defective measles virus and perhaps vaccinia virus are involved. It thus appears that MS may well be a disease of multi-virus etiology. A major team study to elicit the cause of MS, to develop an experimental model to study the disease and to characterize the agents involved is well underway.

Studies of effects of Concanavalin A on slow infections of CNS: (P. Brown, M.D., T. Tsai, M.D, E. French, Ph.D., D.C. Gajdusek, M.D.) Concanavalin A (Con A) is a plant lectin which has been shown to have the property of specific binding to sugar moieities on membranes of cells, protozoans, and viruses. Tumor virologists have shown that transformed cells have as a characteristic property the ability to become agglutinated in the presence of Con A. Normal cells will bind Con A, but do not become agglutinated. Specific binding of Con A to cells infected with viruses has been shown by fluorescent and electron microscopic techniques using conjugated Con A. Enveloped viruses them-

selves are microscopically agglutinated by this lectin. Virus purification has been demonstrated using Con A bound to agarose in affinity chromatography.

We propose to examine cells infected with scrapie, using the property of membrane binding as a probe. There have been clues that carbohydrate content of the membrane of scrapie infected cells may differ from that of normal cells. Scrapie is inactivated to some extent by periodate, an osidant for carbohydrate groups with a specific hydoxyl structure. Scrapie infected cells exhibit both specific and nonspecific alterations in their content of enzymes responsible for metabolism of membrane carbohydrates. The specific binding of Con A and other plant lectins to sugars have revealed different patterns of membrane binding in normal, transformed and virally infected cells. We hope that the possible intercalation of scrapie into the cell membrane will chemically change or physically distort the membrane structure in such a way that binding of Con A will differ from that found in normal cells. We will use the property of altered agglutinability, and visible differences in fluorescent and electron microscopic examination as discriminants.

We have thus far tested primary cultures of normal brain and found that agglutinability is difficult to assess using primary culture cells due to extraneous material present at this level of passage. We are in the process of preparing a fluorescein-conjugate of Con A and of a purified antibody to Con A, in an effort to bypass the less precise cell-agglutination end-point for Con A binding in favor of visualization of the Con A attachment itself.

Significance to biomedical research and the program of the institute: The mission assigned this laboratory in 1962 was to demonstrate that subacute chronic progressive degenerative diseases of the nervous system of man may have infection as their etiology. The data presented in this report conclusively demonstrate that the subacute degenerative encephalopathies, kuru, a heredofamilial disease of man, and Creutzfeldt-Jakob disease, a presenile dementia of man, are both caused by filterable infectious agents which behave like viruses in biological, chemical and physical properties. Significant new and important data collected during the past year have included the detection of the virus of CJD in the liver and lymph node tissue of a patient, the development of disease in a patient 17 months following a single corneal transplant from a donor who died with Creutzfeldt-Jakob disease, and the development of spongiform encephalopathy in subhuman primates inoculated with brain tissue from a patient with familial Alzheimer's disease and a neurosurgeon that had died with a diagnosis of Köhlmeier-Degos disease. These findings have intensified our efforts to determine the distribution of the viruses of kuru, Creutzfeldt-Jakob disease, and familial Alzheimer's disease in the human host since questions of the possible transmission of the disease by blood transfusion, neurosurgical procedures and the neuropathological handling of infected tissues become of great concern.

These studies have further demonstrated for the first time that scrapie, a natural encephalopathy of sheep and goats, which closely resembles kuru and Creutzfeldt-Jakob disease in clinical course and histopathology, can infect

sub-human primates, the cynomolgus monkey, and the squirrel monkey. Our report on the transmission of CJD to the domestic cat and the successful serial passage of the virus from cat to cat, associated with a significant drop in incubation period from 30 months on primary passage to 11 months on first serial passage, is significant to note. It is possible that the original hosts and perhaps even the reservoirs of kuru and CJD may be animals, and emphasize the fact that the scrapie virus with its world-wide distribution may not be inocuous for man. It is worthy of note that a wide variety of "clinical and histopathological types" of CJD, including those described as subacute spongiform encephalopathy, Nevin-Jones disease and Brownell-Oppenheimer disease, have in every instance induced remarkedly similar fatal clinical disease and histological lesions in experimentally inoculated animals with incubation periods that have ranged from 11 to 67 months, and rapid clinical courses that have ranged from <1 to 12 months duration. In addition, we have demonstrated that familial cases of CJD are also transmissible. Our reports on the transmissibility of Creutzfeldt-Jakob disease, previously thought to be a rare disease, have in recent months resulted in the acquisition of brain specimens from more than one hundred new cases--more than doubling the world's previously reported cases. These findings support our hypothesis that other encephalopathies, including the so-called presenile and perhaps even senile dementias of man, Alper's and Pick's diseases, as well as diseases such as multiple sclerosis, amyotrophic lateral sclerosis, Huntington's chorea, progressive supranuclear palsy, chronic encephalitis with focal epilepsy and myoclonus, may be caused by similar agents. More definitive information on the nature of the agents causing kuru and CJD will undoubtedly lead to eliciting the causative agents of these other diseases.

On the other hand our results on studies to determine the etiology of multiple sclerosis clearly support our earlier hypothesis that in some, not all, instances multiple sclerosis is caused by a defective measles virus, a virus much more defective than that associated with SSPE (subacute sclerosing panencephalitis). Nor should other myxo-paramyxoviruses be excluded, since it is entirely possible that like its name, multiple sclerosis will have multiple viruses for some forms of its etiology.

The successful transmission of kuru to rhesus monkeys with an 8 1/2 year incubation period draws attention to the possibility that sub-human primates inoculated with other neurological diseases of man may yet manifest clinical signs of disease associated with exceedingly long incubation periods. Our recent transmission of kuru and Creutzfeldt-Jakob disease to marmosets and adaptation of the virus in this animal, resulting in an appreciable lowering of the incubation period from 36 months on primary passage to 42 days on serial passage, provides the most sensitive test model to date for efficiently characterizing and determining the nature of these viruses. Indeed, these studies have formed the basis of increasing interest and worldwide investigations in the relation between viruses and chronic degenerative diseases of the brain. The isolation of kuru virus has been followed by the demonstration that defective measles virus is associated with subacute sclerosing panencephalitis of children and young adults; the demonstration of a papovavirus etiology for progressive multifocal leukoencephalopathy and the isolation of

SV40 related virus from the brains of at least two patients with this disease; and the demonstration of virus inclusion bodies in patients with chronic epilepsy or epilepsia partialis continue. With our recent demonstration in cell cultures from the brain of a patient with Creutzfeldt-Jakob disease, and with meningioma, a defective virus which in some respects resembles a "conventional" tumor virus, we have opened important and new questions about possible synergistic relationships between different latent infectious agents causing both degenerative encephalopathies and malignant neoplasms of the brain. In collaboration with Dr. Cathala of Paris, we have been able to determine the development of significant changes in the EEG pattern of chimpanzees inoculated with CJD as early as four to five months following injection of the virus. We thus have a sensitive measure of detecting the presence of the virus six to 12 months before the anticipated onset of clinical disease. Such findings are in close agreement with the collaborative studies of Mrs. Elisabeth Beck in Frankfurt, Germany, on the study of sequential neuropathological lesions in spider monkeys inoculated with kuru. Changes consistent with those seen in kuru affected humans and sub-human primates have now been detected as early as three months following exposure to the virus. These later studies support the findings of ealry neuropathological lesions in experimental animals dying of non-neurological intercurrent infection and have resulted in a broadened host range for the study of experimental diseases.

Proposed course: The study of slow, latent and temperate virus infections of man and animals, with particular reference to diseases of the nervous system, but also including chronic and persistent infections due to latent, masked, persistent and infectious agents, will be continued along the lines described in this and previous reports. We shall continue our attempts to transmit additional neurological diseases to suitable laboratory hosts and to isolate and characterize the etiological agents of these diseases. In an effort to arrive at the definitive nature of the viruses of kuru, Creutzfeldt-Jakob disease and scrapie, we shall continue to characterize their biological, chemical and physical properties. Preliminary studies have already been initiated in the area of chemotherapeutic and immunoprophylactic measures that may prove effective in preventing or arresting these diseases in experimental animals. Continued emphasis will be placed on the *in vitro* growth of human and animal tissues in attempts to isolate latent, persistent, chronic, masked viruses which may well be sharing an endosymbiotic relationship with the donor host until some exogenous stress factor activates their pathogenicity *in vivo*. Since the viruses of kuru, CJD and scrapie are now known to persist and probably replicate *in vitro* without lysing the cells, these cultures will be studied in greater detail by electron microscopy, cytogenetic techniques, immunologically for the presence of cell surface antigens and their altered metabolic processes, determined by incorporation of H^3 uridine, H^3 thymidine, and C^{14} glucose. Infectious agents isolated from human and animal tissues obtained at surgical biopsy and early autopsy will be characterized, identified, and classified, and by seroepidemiological techniques we will determine their distribution and significance in human and animal populations. We shall determine their etiological significance in relation to the disease under study. Major emphasis will continue to be placed on a comprehensive study of the human brain transformed cell lines, developed in this laboratory from a patient with Creutzfeldt-Jakob disease.

These studies, in collaboration with the National Cancer Institute, will include purification of the virus and study of the oncogenic, tumorogenic, immunological, chemical and physical properties. Extensive field work will be continued on kuru and related diseases, particularly focusing on the epidemiology and natural reservoirs since cases are still continuing to occur. Studies have already been initiated to determine the presence of cryptic viral genomes in human brain by hybridization studies, employing purified viral nucleic acid probes.

Serial No. NDS (I) - 65 CNSS 1282
1. Intramural Research
2. Central Nervous System Studies Laboratory
3. Bethesda, Maryland

PHS - NIH
Project Report
July 1, 1973 through June 30, 1974

Project Title: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

Sub-Project I. Study of the developmental patterning of the human nervous system (cybernetics of human development).

Sub-Project II: Human evolutionary studies in isolated primitive groups.

Sub-Project III: Studies of isolated Micronesian populations.

Sub-Project IV: Studies of isolated New Guinea populations.

Sub-Project V: Studies of Australian Aborigines.

Sub-Project VI: Studies of isolated New Hebrides and Solomon Islands populations.

Sub-Project VII: Studies of Central and South American Indians.

Sub-Project VIII: Developmental, genetic and disease patterns in primitive populations of Asia, Africa and Polynesia.

Sub-Project IX: Experimental developmental neuropediatrics in infantile programming: an empirical approach to the language of information input into the nervous system.

Sub-Project X: Ciphers and notation for the coding of sensory data for neurological information processing.

Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.

Principal Investigators: D. Carleton Gajdusek, M.D.
Clarence J. Gibbs, Jr., Ph.D.
Paul W. Brown, M.D.

Other Investigators: Michael Alpers, M.D., David M. Asher, M.D., Francoise Cathala, M.D., Peter Fetchko, M.A., Ralph M. Garruto, Ph.D., Ivan Mbaginta'o, Judith Meyer, Donald Rubinstein, Vincent Zigas, M.D.

Serial No. NDS (I) - 65 CNSS 1282

Students: Cynthia Payne, Michael Denisevich, Nina Green, Linda Watson, Brenda Johnson and Steven Ono.

Cooperating Investigators: Timothy Asch, Center for Documentary Anthropology, Brandeis University, Waltham; Dr. P.T. Baker, Department of Anthropology, Pennsylvania State University, University Park; Dr. N. Chagnon, Department of Anthropology, Pennsylvania State University; Dr. Edwin Cook, Department of Anthropology, University of California, Davis; Dr. Cyril C. Curtain, Division of Animal Health, CSIRO, Melbourne; Stephen Brown, M.D., School of Public Health, University of California, Berkeley; Mr. and Mrs. Marek Jablonko, Perugia, Italy; Margaret Mead, Museum of Natural History, New York; Mr. and Mrs. Perry Kennedy, Department of Anthropology, University of Buffalo, New York; Thomas Kiefer, Department of Anthropology, Brown University, Providence; Allan Lomax, Applied Social Research, Columbia University, New York; Dr. Theodore Schwartz, Department of Anthropology, University of California, Los Angeles; Richard Feinberg, Department of Anthropology, University of Chicago; Dr. Denise O'Brien, Department of Anthropology, Temple University, Philadelphia; Dr. C. Hoff, Department of Anthropology, University of Oregon, Eugene; Dr. T. Velasquez, San Marcos University, Lima, Peru; Dr. M. Godelier, Laboratory of Social Anthropology, L'Ecole Pratique Des Hautes Etudes, Paris, France; Dr. Jean Guiart, Museum of Natural History, Paris, France; Dr. Sol Riesenberg, Division of Ethnology, Smithsonian Institution; Dr. Gordon Gibson, Department of Anthropology, Smithsonian Institution, Washington, D.C.; Dr. N.M. Blake, Dr. K. Omoto and Dr. R.L. Kirk, Department of Human Biology, Australian National University, Canberra; Dr. D. Kitchin, Opthalmology Institute, Columbia-Presbyterian Medical Center, New York; Dr. H. Lehman, Department of Biochemistry, University of Cambridge, England; Dr. R. MacLennan, International Agency for Cancer Research, Lyon, France; Dr. R. Rodrique, Wilmington, Delaware; Dr. R.T. Simmons, Melbourne, Australia; Dr. J. Sheridan, Walter and Eliza Hall Institute, Melbourne; Dr. Neville Stanley, University of Western Australia, Perth; Dr. Malcolm Simons, W.H.O. Immunology Centre, Singapore; Dr. Kok Ann Lim, Department of Bacteriology, University of Singapore; Dr. D.L. Vogel, Medical Research Centre, Nairobi; Dr. S.A. Wurm, Australian National University, Research School of Pacific Studies, Canberra; Richard Lloyd, Summer Institute of Linguistics, Papua New Guinea; Dr. R. Hornabrook, Institute of Medical Research, Goroka, Papua New Guinea; Dr. Vincent Zigas, Public Health Department, Papua New Guinea; Dr. R. Tesch, Dr. William Collins and Dr. Leon Rosen, Pacific Research Section, NIAID, Honolulu; Dr. Jan ten Brink, Vught, The Netherlands; Dr. Charles Wisseman, Department of Microbiology, University of Maryland, Baltimore; Dr. Arthur Steinberg, Department of Biology, Case

Western Reserve University, Cleveland; Dr. B. Padget, Department of Microbiology, University of Wisconsin, Madison; Dr. Raymond Roos and Dr. K. Shah, Johns Hopkins Hospital, Baltimore; Dr. David Lang and Dr. Samuel Katz, Duke University Medical School, Durham; Dr. Richard Ferber, Children's Medical Center, Boston; Dr. S. Wiesenfeld, Sacramento Medical Center, University of California, Davis; Dr. Kenneth Brown and Mr. W. Leyshon, NIDR; Drs. F. Neva, L. Miller, W. Collins, NIAID; Dr. Paul MacLean, NIMH; Bishop A. Sowada, Fathers F. Trenkenshuh, D. Gallus, Catholic Mission, West Irian, Indonesia; Dr. T. Gerungen, Dr. J. Sulianti-Sarosa and Dr. K. Dresser, Public Health Department, West Irian; Dr. K. Woodward, Dr. D. Bowdin, Dr. R. Greenough, Dr. P. de Carfort, Dr. S. Reveag and Dr. R. Ratard, Medical Services, New Hebrides; Dr. B. Eyers, Dr. J. MacGregor, Dr. W. Firipae and Dr. R. Lee, British Solomon Islands Protectorate.

Administrative: Marion Poms, Linda Poole, La Donna Tavel.

Man Year:

Total:	38
Professional:	26
Others:	12

Publications: Listed on pages 55 through 59

The projects (I through XI) listed herein and their related studies, as itemized in the Project Reports of previous years, have continued throughout this year and are reflected in the extensive list of publications. Laboratory studies on human biology, genetics and associated molecular biology, immunology, virology and biochemistry are all directed at solving problems chosen from small isolated bands still living in the primitive situation in which these problems may be more appropriately studied than in larger civilized societies. Studies in Micronesia, the New Hebrides and British Solomon Islands were greatly expanded last year during our expedition into those islands on the R/V Alpha Helix of the Scripps Institution of Oceanography. A full prospectus of the studies on these islands was published in the 1973 Annual Report. The specimens and data collected on the expedition have been vigorously studied during this past year, and the results are herein reported along with similar ongoing studies from other remote areas of the world. It is apparent that the collection of such invaluable data and material during the expedition not only address specific problems, but are an extension of well-established, long-range studies in this laboratory.

Disease Patterns in Primitive Isolated Island Populations

To determine the spectrum of diseases found on these islands, with emphasis on those of infectious, genetic or congenital etiologies, and to identify the neurological syndromes present, detailed physical examinations, supplemented as needed by laboratory investigation, were performed on the over three thousand people in New Hebrides and the Solomon Islands. Examination of entire populations were performed in each of the smaller islands and medical surveys made on those with larger populations. The protocol included complete neurological and ophthalmological as well as general medical examination. On several islands, as well as wherever medical problems indicated the need, hematological examination of peripheral blood, thick and thin blood smears for parasite identification, urinalysis, x-ray and electrocardiographic study, cultures for bacteria or viruses and intradermal skin testing with atypical mycobacterial and mycotic antigens were carried out. Heights and weights of all children were recorded and identification photographs were taken for use in future longitudinal growth and development studies.

A survey of neurological diseases was carried out in the Banks, Torres and southern Solomon Islands, in order to determine the most prevalent syndromes and to search for disease foci. The neurological diseases encountered fell into several categories: congenital abnormalities, infectious or post-infectious disease, degenerative disease, peripheral neuropathy and cervical and lumbar spondylosis. The congenital disease frequently resulted in definite structural damage. The examples include hydrocephalus, focal epilepsy (with secondary dystonia involving one hand), spastic paraparesis, hypotonia with hyperreflexia, club feet with hyperreflexia. At least five cretins with mental retardation were seen; two had associated severe myopathy (one cretin on Bellona island had a goiter). The most common sequella of infection seen was lower motor neuron paralysis secondary to poliomyelitis. More than six old cases of poliomyelitis were seen; in each case the initial febrile illness had occurred in the setting of presumed epidemic, since other islanders had died or been similarly affected at the same time. A number of cases of leprosy were seen, one with classical mononeuritis. A case of herpes zoster with radicular pain was found, possibly associated with the high prevalence of varicella, which was active on the islands. The degenerative diseases seen included two cases of parkinson's disease, and two with an olivo-pontocerebellar disease syndrome. The fact that the latter two cases were sporadic and that one patient had a history of febrile illness preceding the onset of neurologic signs, may have indicated that the syndrome was post-encephalitis. One islander had dementia, hyperkinesis, cerebellar signs and hyperreflexia, also possibly post-encephalitis; and two cases of severe optic atrophy, of unknown etiology, were seen.

Peripheral neuropathy occurred in two individuals with a typical Charcot-Marie-Tooth clinical picture, although both cases were sporadic; another had a large component of posterior column disease. One patient, in association with peripheral neuropathy, had cerebellar signs, dementia and a long history of seizures. A common complaint among the islanders was lower back pain, probably reflecting severe spondylosis caused by physical labor. Several

patients with sensory and motor radiculopathies, probably secondary to cervical spondylosis, were seen.

There were certain diseases which were noticeable by their absence: as had been recognized in the past, multiple sclerosis is rare in the tropics and no cases were found. Only one patient with a cerebrovascular accident was seen, reflecting the low incidence of arteriosclerotic disease and hypertension on the islands. No focus of neurologic diseases was seen; in particular, amyotrophic lateral sclerosis, parkinsonism-dementia and kuru-like or other tremor syndromes were not encountered in the Banks, Torres and southern Solomon Islands, and no reports of familial periodic paralysis-like disease such as had been found on Tongariki Island in the New Hebrides.

CNS Defects Associated with Hyperendemic Goiter and Cretinism in Highland New Guinea Populations

These ongoing studies deal with the remarkable focus of endemic goiter wherein the unusually high incidence of enormous goiters is encountered in association with a wide spectrum of all degrees of cretinism and a high incidence of congenital deaf-mutism and feeblemindedness in a contemporary neolithic agricultural society in New Guinea. Previously, we reported the presence of this condition wherein the entire Mulia Dani population, located in the Central Highlands of Western New Guinea, is apparently defective in varying degrees, from barely detectable impairment of intelligence to extreme forms of cretinism and congenital deaf-mutism.

Recently we have redefined the focus of goiter in Western New Guinea and found that protein bound iodine (PBI) and urinary iodine excretion levels in these populations demonstrate a severe iodine deficiency in both goitrous and non-goitrous individuals. Thyroid function studies are currently in progress, but preliminary results suggest no evidence of hypothyroidism in the population, although they are on deficient iodine intakes as low as 20 ug/day. This leads us to reaffirm that iodine deficiency is the primary cause of endemic goiter and cretinism in New Guinea highland populations, and that the familial sporadic goiter and cretinism in small islands and coastal populations (our data from the Solomon Islands expedition) is more likely genetically determined. We are also studying varying uses of iodine-binding roughage such as the sweet potato leaves, a dietary staple in Western New Guinea, which may be the deciding factor in determining the emergence of the clinical problem in these highland peoples.

Population Genetic Studies in Relation to Congenital Defects and Heredofamilial Diseases in the Isolated Micronesian, New Hebrides and British Solomon Islands Populations

A wide search for heredofamilial diseases and congenital abnormalities was carried out and genealogies collected on island families. These data taken together with laboratory data on pleomorphisms in erythrocyte surface

antigens, red cell enzymes and serum proteins, genetic data on finger and palm prints, anthropometric measurements and color blindness tests, and medical surveys for such genetic traits as albinism and pigmented rings about the optic disc, are all of importance in studying the genetic structure of populations from the different islands with relation to gene drift and with respect to possible relationships as indicated by their genetic similarities and dissimilarities. The data will be used to search for linkage between genetic constitution to the occurrence of various diseases or morphological traits.

Blood group genetic factors and hemoglobinopathies: Clotted red cells are analyzed for blood groups ABO, MNS, Rh, Kell, Duffy, Diego, Wright and Lewis, by Dr. M. Simons of W.H.O. in Singapore, Dr. K. Brown and W.C. Leyshon at the NIDR, NIH, and by Dr. R. Kirk at the Australian National University, Department of Human Biology. In addition, hemoglobin electrophoresis, in search for hemoglobin A Tongariki, as well as other hemoglobin variants, is being carried out by Dr. Kirk. The data, together with data on other polymorphic loci, will be used to determine genetic relationships and distinctions between the various island population studies. Data on the distribution of hemoglobin A Tongariki will be invaluable to the study of genetic drift and migration in the New Hebrides, as well as data on other rare alleles among the red cell enzymes or other systems.

Red cell enzyme pleomorphisms: Red cell clots were obtained on over three thousand individuals in the islands of New Hebrides and the British Solomon Islands. These samples are currently being analyzed in Dr. Kirk's section at the Australian National University for polymorphisms at twenty different red cell enzyme loci. The results will be used in conjunction with other genetic information to analyze the degree of genetic isolation in the island populations, and in our studies on genetic heterogeneity and gene drift, as well as in genetic distance summaries. They are of great value in indicating and supported suspected migration patterns, and will be used with other data on pleomorphisms to calculate genetic distance and a search for their linkage to various diseases.

Polymorphisms of serum protein factors: More than threee thousand samples are being analyzed by Dr. Kirk for serum protein factors known to be polymorphic. These factors include transferrin, haptoglobin, albumin, group-specific protein (Gc), Gm and Inv allotypes of human immunoglobin G (IgG). A portion of each serum specimen will be kept frozen at $-70^{\circ}C$ for future analysis of yet to be discovered polymorphisms. These data will further amplify the forces of genetic drift, migration and possible selective forces acting on these isolated populations suspected of having as high a coefficient of kinship as can be found anywhere in the world.

Dermatoglyphics: Fifteen hundred subjects were palm and finger printed on both hands for dermatoglyphic analyses by Chris Plato at the NICHD. The data will be used to search for phenotypic differences in palm printing pattern and finger print pattern distribution and in ridge counting, in order to find evidence for relationship or genetic distinctions among the various island populations studied. An attempt is also being made to use dermatogly-

phic data in genetic distance studies and by employing erythrocyte antigens, serum protein and red cell enzyme pleomorphisms for the study of genetic heterogeneity and gene drift by genetic distance techniques.

Genetic distance studies between island groups: Data on close to thirty polymorphic loci, as well as dermatoglyphic phenotypes and kinship patterns, will be used in genetic distance analyses of the island populations surveyed. Genetic heterogeneity as well as coefficients of kinship will be determined. Graphic analyses, including the construction of genetic networks and principal components analysis, will also be attempted. Distinctions between kinship, determined by bioassay (from polymorphism data) and that determined by migration patterns, dermatoglyphics and kinship patterns obtained from history, is currently being contrasted.

Kinship systems and marriage and migration patterns: Medical documentation for the entire three thousand or more patients who were examined and bled includ name, age, sex, village and island, marital status, name of patient's spouse, names and villages and islands of patient's parents, living children, and the reproductive history of the women, as well as travel and medical histories for each patient. Particular care was given to tracing the genealogical lines resulting from foreigners and immigrants to the islands during the last two or three generations. Individuals were questioned as to their assigned membership in exogamous, named social groupings (clans or lineages) and an attempt was made to elaborate the socially prescribed patterns of marriage among those groupings. This documentation makes possible a reconstruction of the genealogical and residential patterns on each island, and thus provides both an elucidation of the pattern of non-random mating and a measure of population dispersion among these isolated populations.

South American Indian Studies. Colombian Indian studies: Red cell samples from persons belonging to four Amerindian linguistic groups in Colombia were investigated for genetic variants in eight blood group systems, ten red cell enzymes, and four serum protein systems. The two groups studied are the Noanama of the Rio Siguirisua, and the Rio Docampado on the Pacific lowlands; and the Cogan, Ingano and Siona Indians of the Upper Rio Putumayo and its tributaries to the east of the Andes. Only blood group O, KP(b+), K, Di(a+) frequencies were high in all groups, with marked variation between groups in the MNS, Rh, P, Lewis and Duffy systems. Polymorphisms for serum proteins and red cell enzymes were present only in the red cell acid phosphatase, phosphogluconate (locus-1) and haptoglobin systems. In a few individuals there was evidence of the presence of an 'atypical' component in the LDH system. Comparison with published data on red cell enzyme and serum protein groups for other South American Indians show the Colombian populations studied most closely resemble the Cayapo of Brazil.

Peruvian and Bolivian Indian studies: Quechua Indians living at various altitude gradients from sea level to above 14,000 ft. were studied for variation in the ABO, Rh blood group systems, dermatoblyphic patterns, as well as such gene markers as digital interlocking, lingual rotation and PTC tasting ability. Our studies to date suggest that

irrespective of altitude, all groups maintain a relative genetic homogeneity. This is apparently due to the moderate level of gene flow as estimated by migration rates into these groups by surrounding Quechua populations. This is further supported by ethnohistorical evidence which does not indicate that the highland Quechua populations were completely isolated from each other or from coastal populations.

Paraguayan Indian studies: Genetic studies of 540 Paraguayan Indians from nine tribal groups, and 51 Mennonites were studied for ANO, MNSs, P, Rh, Kell, Lewis, Duffy and Diego blood groups. Serum immunoglobins and haptoglobins, G6PD deficiency, and thalassemia trait were also studied. Differences among groups studied and between Paraguayan and other Amerinds emphasize the importance of genetic drift and founder principle. Abandonment of their tribes by mixed blood offspring is partially responsible for apparent genetic purity and homogeneity of groups.

Physiological and Hematological Responses of Peruvian Quechua Indians to Chronic Hypoxic Stress

The most important physiological processes in the adaptation of native Andean man to his environment are those which serve to minimize tissue hypoxia. We studied hematological characteristics, including red and white cell counts, hemoglobin concentrations, hematocrits, red cell indices, reticulocytes and differential leucocyte counts in more than three hundred Quechua Indian children, adolescents and adults living at a mean altitude of 14,000 ft. in the Peruvian Andes. Our results suggest that Quechua Indians who live a traditional life as subsistence farmers and herders do not show the severe polycythemia as has been previously reported by earlier works and reviews. At barometric pressures of two-thirds to one-half that of sea level, increasing the O_2 content of arterial blood by increasing red cell mass, would appear to help alleviate tissue oxygen deficit. In severe polycythemia, however, the adaptive benefits may be minimized because of resultant increase in blood viscosity, thereby increasing the work-load on the cardiopulmonary system. Highland Quechua Indians at these altitudes probably still require a low level of polycythemia, albeit by no means as severe as had been generally proposed by earlier reports. Continuing studies will deal with the effects of strenuous physical activity on the hematological characteristics, as well as detailed erythrokinetic studies of these highland peoples, to further augment our knowledge of how populations adjust biologically as well as culturally to severe chronic environmental conditions.

Seroepidemiological Investigations for Virus, Rickettsial, Bacterial, Myocotic, Protozoal and Helminthic Diseases and Virus Isolations

Sera were collected from three thousand subjects, or over 60% of the population, in the islands intensively studied. In islands given our particular attention, such as Merig in the New Hebrides and Anuta in the Solomon Islands, over 90% of the population were bled. Over the course of several years the sera will be investigated for a wide variety of infectious disease

antibodies. Techniques of complement fixation, hemagglutination inhibition, fluorescent antibody, neutralization, indirect hemadsorption inhibition, and agglutination will be used to identify viral and rickettsial antibodies. Antibody testing for infection with various specific protozoa, bacteria, helminths and fungi will be conducted. Age incidence of acquisition of immunity or first contact with each microbe will be plotted to increase our understanding of the pattern of spread of infectious diseases in these population isolates.

Particular attention is being given to arbovirus infections, in view of the ecology of these islands and the paucity of species of natural hosts and vectors which are found. Continuation of studies on the mode of transmission of toxoplasmosis will be a major interest. Since none of the people had contact with monkeys, or vaccines prepared in monkey tissue culture, the antibody survey of these populations for antibodies to Simian viruses is of particular significance. Antibody patterns to the various papoviruses isolated from human brain of patients with progressive multifocal leucoencephalopathy (SV40, J.C. virus and the related BK virus) to determine the frequency and age incidence of contact with these agents as possible clues to their epidemiology in man, are being quickly determined. Similarly, in view of the importance of our understanding of the carrier state for herpesviruses in man and the possible role of these agents in the genesis of cancer and other chronic diseases, antibody acquisition patterns for herpes hominis types 1 and 2, Epstein-Barr virus, cytomegalovirus, varicella and herpes zoster are under study. Australian antigen and Australian antigen antibody will be determined, also antibody response to respiratory syncytial virus and the kuru foamy viruses of man, chimpanzees, monkeys and non-primates.

A large number of specimens for virus isolation have been collected; they include, principally, throat washings, fecal and some urine specimens, and are now maintained at -70°C while being slowly processed for the presence of viruses during this next year.

Seroepidemiology of Human Papovaviruses: These studies are attempting to examine antibodies to a new strain of human papovavirus, BK virus, and to describe features of the epidemiology of this virus in isolated human populations which may provide insight into its association with disease, as well as to correlate in selected sera the presence of BK antibodies with those of SV40 and J.C. virus, two other human papovaviruses associated with progressive multifocal leucoencephalopathy (PML).

Remote or unusual populations were tested, representing over a thousand serum specimens from Micronesia, Melanesia, isolated Indian tribes in South America, aboriginal groups in Australia and Malaya, Alaskan Eskimos, isolated villages in Iceland, and a diverse group of the Middle East. Antibody to BK virus was measured by conventional HI, FA and neutralization tests. A plaque reduction neutralization test was used to measure antibody to SV40.

Normal populations from the Westernized societies of England, Finland and the United States (Maryland) have been reported to have antibody prevalence of 60 to 70%. In these groups antibody is acquired throughout childhood and prevalence reaches a peak in the prepubertal years; older age cohorts are reported to have a diminished percentage of antibody positivity. In most of the populations we examined, despite their isolation, we confirm a high level of exposure to BK virus. Prevalence ranged from 60% in Western New Guinea to 90% in isolated islands of Oceania. Unlike acute respiratory infections, such as mealses, in which a short incubation period and a cyclic pattern of epidemic spread has spared specific age cohorts of our remote groups from exposure to the agent, we found contact with BK virus to be geographically and temporally ubiquitous.

In these diverse normal populations we were unable to describe any unusual distribution of antibody by the criteria of sex, geographic area, degree of population dispersion, presence of vectors, or hygienic standards. The age pattern of antibody acquisition in our groups closely parallels that previously reported. Children rapidly acquire antibody and have nearly all experienced infection by adolescence. We were unable to find in any of our populations a decline in the percentage of antibody positive individuals in the older age groups. Rather, a plateau of high antibody positivity once reached in adolescence is maintained throughout adulthood. We found several 'virgin' populations in the very remote areas of Paraguay and Brazil.

Sera from chimpanzees, monkeys and rodents were also tested; none of these animals were found to have antibody to BK virus.

A good correlation was found between levels of HI and FA antibody to BK virus. Neutralizing antibody to SV40 occurred almost exclusively in individuals with high levels of HI antibody to BK virus, suggesting far more serological cross-reaction in humans than had been previously supposed.

The association of papovaviruses of the polyoma family with human disease other than PML has yet to be established. BK virus can be shed in the urine of immunosuppressed renal transplant patients, but no spontaneous infection has been documented. The role of viruses of this group in experimental oncogenesis and the hypothesis that a papovavirus may be important as a participant in the dual viral infection in the pathogenesis of subacute neurologic disease, establish the need for investigation of the range of contact of these viruses in human populations. Our results, demonstrating a consistent high level of contact with the virus in diverse groups of people, and an absence of association with disease, suggest that infection with BK virus is widespread and that it may be commensal.

Screening of patients with various neurologic diseases and a similar sero-epidemiologic study for J.C. virus, is in progress. Clinical studies of normal children in susceptible age groups to correlate sero-conversion to these agents with disease states is also planned.

Seroepidemiology of Herpesviruses: Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) antibodies have been studied among remote Micronesia, New Hebrides, British Solomon Islands and New Guinea populations. CMV and EBV members of the herpes group viruses have been implicated in the establishment of persistent and/or latent infections. Most populations studied in Western Europe, Great Britain and the United States exhibited the gradual acquisition of these virus antibodies. We have, however, found that among the isolated South Pacific populations we studied, there was an early acquisition of CMV and EBV antibodies. In some cases positive titers reached 100% by mid-childhood. In all groups studied nutrition is marginally adequate and hygiene primitive. There exists a great deal of close personal contact. Many children, often from several families, may carry, handle and play with a given infant and many adults will similarly handle one small child. Furthermore, food is premasticated for small babies, often by several individuals, and considerable mouth play, particularly with infants was observed. These interactions probably enhance the early acquisition and spread of herpesviruses which are relatively labile and spread through direct personal contact, rather than by aerosols or fomites. We suspect that the much lower rate of acquisition of EBV and CMV antibodies previously documented in Western cultures, living in a temperate climate, is related to lesser personal contact than to improved hygiene and sanitation.

The impact of early acquisition to these viruses upon the development of chronic infections and any associated late sequelae remains to be established. Persistent infection acquired early in life probably prevents the appearance later of disease, reflecting primary infections including those acquired during pregnancy, which may be associated with fetal malformation and injury. Further studies of CMV and EBV are currently in progress, as are studies of Herpes 1 and 2 in these same populations.

Arboviruses in isolated Micronesian and Melanesian populations: Extensive laboratory studies are currently in progress to determine the epidemiological patterns of Group A and Group B arboviruses in the South Pacific area. These include studies of the Micronesian islands, New Hebrides, the British Solomon Islands, Papua New Guinea, Western New Guinea and the Indonesian islands and Malaysia. Clinal variations in Group A and Group B arbovirus patterns are of major interest, as are virgin soil populations and hyperendemic areas.

Plaque reduction neutralization tests were performed on human sera from a number of the isolated South Pacific populations. Selected sera from each population were screened at a 1:10 dilution against five Group A arboviruses (Chikungunya, Ross River, Getah, Sindbis and Bebaru). Sera producing 90% plaque reduction were recorded as positive. This method was employed because it is much more specific than the hemagglutination-inhibition tests, the latter of which are difficult to interpret due to the rather broad cross-reactivity among the various Group A arboviruses.

Preliminary results indicate that the prevalence of Ross River neutra-

lizing antibodies among inhabitants of New Guinea and other nearby islands is quite high and in some cases very high, suggesting a recent epidemic of Ross River infection. Attempts will be made to test sera by neutralization tests against several Group B viruses, although our results indicate that there is broader cross-reactivity among dengue, Japanese B encephalitis, and Murray Valley encephalitis than with Group A agents.

Survey of Skin Test Sensitization to Tuberculosis, Atypical Mycobacteria, Coccidioidin and Histoplasmin

Because of the high incidence of tuberculosis in the populations on some of the islands studied, an attempt was made to recognize all cases of tuberculosis early and to evaluate the true extent of the problem. The possibility that atypical mycobacteria may be contributing to the pattern of chronic pulmonary diseases seen was thought to be great. Intradermal skin tests, using seven mycobacterial antigens (PPD-A, PPD-B, PPD-F, PPD-G, PPD-S, PPD-T and PPD-Y), coccidioidin and histoplasmin, were performed on three hundred subjects of all ages on three of the islands: Merig, in the Banks Islands; Loh, in the Torres Islands; and Anuta, the most remote of the Polynesian outliers in the British Solomon Islands. On Anuta, sixty chest x-rays were taken on islanders who could reach the ship and who had been skin-tested with all nine antigens. All subjects studied had had complete physical examinations.

No person on any island gave a positive reaction to either of the two fungal antigens, which is consistent with what we found on other islands throughout the Pacific region. The prevalence of positive reactions to PPD-S, the international standard antigen to test human tuberculosis, was under 15% in both of the New Hebrides islands, but over 50% in Anuta in the Solomons. Considerable cross-reactivity was apparent with the other atypical mycobacterial antigens tested, but in no case did the degree of positivity seen in the population as a whole indicate that primary infection was due to an atypical bacterium. One of the most interesting observations was that the New Hebrides island populations, which had received BCG vaccination in previous years, had the lowest rate of skin positivity, whereas the unvaccinated population of Anuta, with no clinically apparent tuberculosis, had the highest rate of skin positivity. One explanation would be a racial difference, Anuta being Polynesian, and Merig and Loh being Melanesian, but further study will be required to determine the actual reasons for this anachronism.

Chest Roentgenographic Survey of the Islands

In order to determine the extent and characteristics of known and unknown pulmonary disease in the islands studied, over four hundred chest x-rays were taken by means of a portable x-ray unit aboard the R/V Alpha Helix. These studies included screening of about half the population on Anuta, one of the most remote islands, and surveys of various villages and clinically ill individuals on other islands. Also carried out was the screening of almost

all the students from many different southern Solomon Islands and the examination of many patients at the government hospital on Santa Cruz. These studies will be useful in determining what patients should be evacuated for treatment of tuberculosis, as well as for aiding our understanding of the distribution of disease, such as tuberculosis and atypical mycobacterial infection throughout the islands. Correlation of chest x-ray findings with PPD and atypical mycobacterial and mycotic antigen skin sensitivities will be performed. In addition, some individuals were found to have roentgenographic evidence of cardiovascular disease. Two cases of probable initial stenosis, one case of pulmonary stenosis, and several people with cardiomegaly of undetermined origin were discovered. An incidental finding was the occurrence of calcium on the aortic arch in about the same frequency as occurs in the United States, suggesting that the absence of vascular disease syndromes in this population is not the result of an absence of atherosclerosis.

Parasitology: Malarial and Filarial and Intestinal Parasite Studies

Fifteen hundred thick and then blood smears were taken on the island populations for evaluation of the incidence of parasitemia for malaria and filaria. Over two thousand people were examined for clinical evidence of malaria and for the presence of an enlarged spleen. Stool specimens in appropriate holding media provided a means for assessing the levels and kinds of helminthic and amoebic infectation in the populations in New Hebrides and the British Solomon Islands. Hematological studies, including hemoglobin determinations and differential blood counts also provided an index of anemia and of eosinophilia. Fluorescent antibody tests for malaria were performed on selected population samples and correlated with the results of blood smear evaluations.

Results of clinical examinations indicated a rather low level of malarial illness throughout the islands, but the spleen index, particularly the distribution of enlarged spleens predominantly in the younger age groups, indicated a high level of malarial infection consistent with a holoendemic malarial region. Serological examination of the population confirmed the widespread existence of malarial infection, predominantly with P. falciparum and P. vivax. Hematological studies on the selected islands (Merig in the Banks Island, Hiu in the Torres Island, and Anuta in the Solomon Islands) further confirmed these two species as the prevalent ones and also showed that the burden of malaria on these islands was not responsible for any significant incidence of anemia.

Certain islands in the Solomons were shown to be malaria-free, and others to harbor only a single species of malaria. This information will be used to 'standardize' the serological tests used to detect malarial antibody which until now has been deduced only from human prison volunteer studies in this country. It is interesting also that the antibody levels in these isolated populations are among the highest reported in the world, despite the relatively low prevalence of clinical malaria. Studies are currently under way to determine the significance of this observation.

A W.H.O. malaria suppression program is already under way in the Solomon Islands, and a similar program is envisioned for the near future in the Banks and Torres Islands. Our results, which comprise the most comprehensive study of the problem in this area, will be invaluable to physicians and scientists converned in these programs.

Film Archive Studies

The collection, documentation and research utilization of cinema film on ethnopediatrics, human biology and ecology, cybernetic programming of the central nervous system, and child behavior and development, have continued during the period reported. Several major film corpi have been acquired from anthropologists working among the Anga (Kukukuku) of New Guinea, and among the Waunan Indians of the Rio Siguirisua in Colombia. These have been thoroughly documented and cross-referenced with relevant publications and field notes, and provide valuable backgrouns to other studies of disease patterns and human biology in those areas.

This year there has been a continuation of our collection of material in Micronesia, with the inauguration of studies in Sonsorol and Pulo Anna, and continued work in Ulithi and Fais. Investigations in Western New Guinea are also being pursued this year, with special attention to central nervous system diseases and ethnopediatric patterning of the developing nervous system. The films obtained in these studies are in the process of being made into Research Films, incorporating the carefully documented medical and ethnological information into the body of the finished films. Considerable progress has been made in eliminating substantial backlog of cinema material that had not yet been converted to Reseaach Films. Several Research Special Films have been finished or are in progress. These constitute comprehensive collections of all the available films on a chosen subject, arranged in chronological order, and fully dated and documented. Their use by researchers and collaborators is an invaluable aid to those unable to obtain first hand knowledge. These include Special Films on kuru, tremor syndromes occuring in the Eastern Highlands of New Guinea, goiter in Western New Guinea, nutrition in the kuru region, Anga (Kukukuku) salt manufacture, longlong (a psychiatric disorder occurring in New Guinea), and Anga sex avoidance behavior. Another type of film that has been produced this year has been the Special Demonstration Film. There has been an increasing demand for film that can illustrate to clinicians and other researchers some of the rather esoteric disease problems with which we work. We have, therefore, produced films from the Research Film collection (without disturbing or altering the Research Films themselves) a small number of demonstration films on the following subjects: kuru in humans, kuru in chimpanzees, scrapie (both natural and experimental) in sheep and mice, and encephalopathy in mink, Creutzfeldt-Jakob disease in chimpanzees, and a film on human C-J disease is in progress.

Work on the cinema record from the expedition on the R/V Alpha Helix has continued. These Archives amount to more than 11,000 feet of 16mm color film. Motion picture documentation was undertaken for several purposes: 1) cinema records were made on patients with neurological and other movement

disorders to permanently record and permit the further analysis of their conditions; 2) as a supplement to the medical survey and population genetic studies; they provide the ethnographic background essential to the interpretation of epidemiological and human biological observations and laboratory findings; 3) medical problems other than neurological, which could be effectively recorded photographically, including elephantiasis, other filarial infection syndromes, albinism, contenital deformations, goiter and cretinism, and various skin diseases; 4) as part of the Study of Child Growth and Development cinema was used to record behavior patterns and early childhood learning; and 5) in accordance with the wishes of the Scripps Institution and the National Science Foundation, the field procedures of the expedition and research use of the Alpha Helix facilities in dealing with island populations and handling of medical problems were also filmed.

The entire 11,000 feet of motion picture film will be fully documented with supplementary material and will become Research Films of the Study of Child Growth and Development, and Disease Patterns in Primitive Cultures of the National Institute of Neurological Diseases and Stroke. Eleven of the expedition participants used one or more still cameras, documenting extensively every area of their work. There are more than eight thousand photographs, 90% of them in color. They fall into the following categories: 1) documentation of medical and genetic problems encountered, including filarial adenopathy, elephantiasis, tropical pyomyositis, tropical ulcers, trachoma, leprosy, gangosa yaws, goitrous cretinism, tinea versicolor and corporis, molluscum contagiosum, pedunculated warts, albinism and partial albinism, various congenital anomalies with and without mental retardation, club feet, reduction deformities, supernumerary digits, external ear deformities, diastasis recti, ptosis and atrophied extremities and post-poliomyelitis atrophy and paresis; 2) still photographic record of behavioral importance in the study of child growth and development, to be used in longitudinal studies; 3)identity photographs on individuals from whom we have a blood sample, for later correlation with human biological results and growth studies; 4) ethnographic background material to aid in the interpretation of genetic and medical findings and a general understanding of their cultural setting; and 5) photographs documenting the natural history and ecology of the islands, for use in epidemiological studies and on disease ecology studies.

Collection and documentation of ethnographic artifacts: Several hundred tools, household articles and ceremonial carvings were collected in the villages and district centers which the expedition visited. A principal aim of the photography during the expedition was to supplement this collection with a photographic record of several thousand artifacts in their village setting, particularly with reference to architecture of the houses and settlement patterns, the use of indigenous tools, native art styles, tattoo patterns, canoe construction, traditional dress and the technology of gardening, irrigation, and food preparation. The collection and documentation of these artifacts will provide an ethnographic picture of the living conditions and the dietary habits of the islanders and will be important as background cultural

data for studies of child rearing and disease patterns on the islands. The documentation of cultural styles of art and technology will also be useful as supplementary data to the medical studies of genetic distance among the island populations.

Child drawings and the recording of music: Several hundred drawings were collected from children in most of the island groups studied. We have recorded the name, age and sex, and village residence of each child artist. Each child's depiction, frequently in a culturally stylized manner of his island world and activities within the village and on sea, provide a useful and unique document for the study of child growth and development. These drawings are being used together with the large collection of drawings from different cultural and linguistic groups in Melanesia, especially New Guinea, Polynesia and Micronesia, which we have accumulated from children in low-acculturated societies over the past twenty years for the study of culturally determined symbolic styles and patterns of cognitive function.

Ten hours of island music, dance and communal singing were recorded on magnetic tape in the islands of New Hebrides and Solomon Islands and will be used principally as supplementary material to the corpus of cinema and flash still photography. On several islands we had the opportunity to make high quality recordings of traditional Polynesian and Melanesian dance-songs and commemorative chants which will become interesting ethnographic supplements to the studies of human biology of these islands.

Publications

Alpers, M.P., and Gajdusek, D.C. Problems of so-called "recoveries" and of differential diagnosis in kuru. N. Engl. J. Med., in press.

Alpers, M., Gajdusek, D.C., and Gibbs, C.J., Jr. Subacute spongiform encephalopathies, transmissible degenerative diseases of the nervous system. In Kakulas, B. (ed.): Clinical Studies in Myology, Part 2. Proceedings of the Second International Congress on Muscle Diseases, Perth, November, 1971. I.C.S. No. 295, Amsterdam, Excerpta Medica, pp. 3-7.

Asher, D.M., Gajdusek, D.C., and Gibbs, C.J., Jr. Recent progress in the study of subacute spongiform encephalopathies. Vestn. Akad. Nauk SSSR 2, 34-43, 1973.

Asher, D.M., Gibbs, C.J., Jr., David, E., Alpers, M.P., and Gajdusek, D.C. Experimental kuru in the chimpanzee. Physical findings and clinical laboratory studies. In Montagna, W., and McNulty, W.P. (eds.): Nonhuman Primates and Human Diseases. Fourth International Congress on Primatology. Basel, S. Karger 4: 43-90, 1973.

Asher, D., Gibbs, C.J., Jr., Lang, D.J., and Gajdusek, D.C. Persistent shedding of cytomegalovirus in the urine of healthy rhesus monkeys. Proc. Soc. Exper. Biol. Med. 145: 794-801, 1974.

Asher, D.M., Hooks, J., Amyx, H., Barki, H., Luber, N., Gibbs, C.J., Jr., and Gajdusek, D.C. Persistent shedding of adenovirus in urine of healthy chimpanzees. J. Infec. Immun., in press.

Beck, E., Daniel, P.M., Asher, D.M., Gajdusek, D.C., and Gibbs, C.J., Jr. Experimental kuru in the chimpanzee: a neuropathological study. Brain 96: 441-462, 1973.

Beck, E., Daniel, M.P., and Gajdusek, D.C. Human kuru: a pathological study of 40 brains. Brain, in press.

Benfante, R.J., Traub, R., Lim, K.A., Hooks, J., Gibbs, C.J., Jr., and Gajdusek, D.C. Immunological reactions in kuru: attempts to demonstrate serological relationships between kuru and other known infectious agents. Am. J. Trop. Med. Hyg. 23: 477-488, 1973.

Blake, N.M., Omoto, K., Kirk, R.L., and Gajdusek, D.C. Variation in red cell enzyme groups among populations of the Western Caroline Islands, Micronesia. Am. J. Hum. Genet. 25: 413-421, 1973.

Bobowick, A.R., Brody, J.A., Matthews, M.R., Roos, R., and Gajdusek, D.C. Creutzfeldt-Jakob disease: a case control study. Am. J. Epidem. 98: 381-394, 1973.

Brown, P., Cathala, F., and Gajdusek, D.C. Further studies of viral antibodies in the cerebrospinal fluid of patients with multiple sclerosis: vaccinia and parainfluenza type 1. Proc. Soc. Exper. Biol. Med. 143: 828-829, 1973.

Brown, P., and Gajdusek, D.C. No mouse PMN leukocyte depression after inoculation with brain tissue from multiple sclerosis or spongiform encephalopathies. Nature 247: 217-218, 1974.

Brown, P., Sarragne, M., Chany, C., Gibbs, C.J., Jr., and Gajdusek, D.C. Tissue antagonists of interferon in normal and kuru or Creutzfeldt-Jakob infected primate brain. C.R. Acad. Sci. Paris, in press.

Cathala, F., and Brown, P. La maladie de Creutzfeldt-Jakob. Une "Encephalopathie spongiforme a virus." Pathol. Biol. (Paris) 21: 299-308, 1973.

Cathala, F., Court, L., Rohmer, F., Gajdusek, D.C., Gibbs, C.J., Jr., and Castaigne, P. Experimental transmission of Creutzfeldt-Jakob disease to a chimpanzee, with an electroencephalographic study using indwelling electrodes. In Subirana, A., Espadaler, J.M., and Burrows, E.A. (eds.): Proceedings, X International Congress of Neurology. Amsterdam, Excerpta Medica, in press.

Chou, S.M., Roos, R., Burrell, R., and Harley, J. Subacute focal adenovirus encephalitis. J. Neuropathol. Exp. Neurol. 32: 34-50, 1973.

Curtain, C.C., Gajdusek, D.C., O'Brien, D., and Garruto, R.M. Congenital defects of the central nervous system associated with hyperendemic goiter in a neolithic highland society of Western New Guinea. IV. Serum proteins and haptoglobins, transferrins and hemoglobin types in goitrous and adjacent non-goitrous Western Dani populations. Hum. Biol. 46: 1974, in press.

Ferber, R.A., Wiesenfeld, S.L., Roos, R., Bobowick, A.R., Gibbs, C.J., Jr., and Gajdusek, D.C. Familial Creutzfeldt-Jakob disease. Transmission of the familial disease to primates. In Subirana, A., Espadaler, J.M., and Burrows, E.A. (eds.): Proceedings, X International Congress of Neurology. Amsterdam, Excerpta Medica, in press.

Gajdusek, D.C. The characteristics of human subacute spongiform virus encephalopathies. In Biochemical Society Transactions 1: 1058-1061, 1973. Oxford, University of Oxford.

Gajdusek, D.C. Slow and latent viruses and the aging nervous system. In Maletta, G. (ed.): Survey Report of the Aging Nervous System. NICHD Publication, Government Printing Office, Washington, D.C., in press.

Gajdusek, D.C. Kuru and Creutzfeldt-Jakob disease. Experimental models of non-inflammatory degenerative slow virus diseases of the central nervous system. Ann. Clin. Res. (Helsinki) 5: 254-261, 1973.

Gajdusek, D.C. Pneumocystis carinii as the cause of human disease: historical perspective and magnitude of the problem. Introductory remarks. In Robbins, J.B., and DeVita, V.T. (eds.): Symposium on Pneumocystic Carinii Infection. Monograph Series, JNCI, 1973, in press.

Gajdusek, D.C., Alpers, M.P., and Gibbs, C.J., Jr. Kuru: epidemiological and virological studies of a unique New Guinean disease with wide significance to general medicine. In Hornabrook, R.W. (ed.): Symposium on Kuru. In press.

Gajdusek, D.C., and Garruto, R.M. The focus of hyperendemic goiter, cretinism and associated deaf-mutism in Western New Guinea. In Johnson, F.J., and Watts, E. (eds.): Biosocial Interrelations in Population Adaptation. The Hague, Mouton Publishers, 1974, in press.

Gajdusek, D.C., Garruto, R.M., and Dedecker, R. Congenital defects of the central nervous system associated with hyperendemic goiter in a neolithic highland society of Western New Guinea. V. A note on birth weights and infantile growth rates in the Mulia populations. Hum. Biol. 46: 1974, in press.

Gajdusek, D.C., and Gibbs, C.J., Jr. Kuru and the virus dementias. In Helman, A., Oxman, M.N., and Pollack, R. (eds.): Biohazards in Biological Research. New York, Cold Spring Harbor Press, 1973, pp. 288-299.

Gajdusek, D.C., and Gibbs, C.J., Jr. Transmissible virus dementias. In Fuente, R. de la, and Wiesman, M.N. (eds.): Proceedings of the V World Congress of Psychiatry. Series No. 274, pp. 1228-1234, 1971. Amsterdam, Excerpta Medica.

Gajdusek, D.C., Gibbs, C.J., Jr., Earle, K., Dammin, G.J., Schoene, W., and Tyler, H.R. Transmission of subacute spongiform encephalopathy to the chimpanzee and squirrel monkey from a patient with papulosis atrophicans maligna of Kohlmeier-Degos. In Subirana, A., Espadaler, J.M., and Burrows, E.A. (eds.): Proceedings, X International Congress of Neurology. Amsterdam, Excerpta Medica, in press.

Garruto, R.M. Hematology. In Baker, P.T., and Little, M.A. (eds.): Man in the Andes: a Multidisciplinary Study of High Altitude Quechua. Chapter 15, Synthesis Volume. U.S. International Biological Program, Human Adaptability, in press.

Garruto, R.M., Gajdusek, D.C., and ten Brink, J. Congenital defects of the central nervous system associated with hyperendemic goiter in a neolithic highland society of Western New Guinea. III. Serum and urinary iodine levels in goitrous and adjacent non-goitrous populations. Hum. Biol. 46: 1974, in press.

Garruto, R.M., Hoff, C., and Baker, P.T. Phenotypic variation in four gene marker systems among the Quechua of Southern Peru. Hum. Biol., in press.

Gibbs, C.J., Jr. and Gajdusek, D.C. Biology of kuru and Creutzfeldt-Jakob disease. In Zeman, W., and Lennette, E.H. (eds.): Slow Virus Diseases. Baltimore, Williams and Wilkins, 1974, pp. 39-48.

Gibbs, C.J., Jr., and Gajdusek, D.C. Cell-virus interactions in slow infections of the nervous system. In Schmitt, F.O., and Worden, F.G. (eds.): The Neurosciences. Third Study Program. Cambridge, Massachusetts, M.I.T. Press, 1974, pp. 1025-1041.

Gibbs, C.J., Jr., and Gajdusek, D.C. Experimental subacute spongiform virus encephalopathies in primates and other laboratory animals. Science 182: 67-68, 1973.

Herzberg, L., Gibbs, C.J., Jr., French, E.L., Asher, D.M., and Gajdusek, D.C. Slow, latent and chronic viral infections of the central nervous system. In Greenwalt, T.J., and Jamieson, G.A. (eds.): Transmissible Disease and Blood Transfusion. New York, Grune and Stratton, in press.

Hooks, J., Gibbs, C.J., Jr., Chou, S., Howk, R., Lewis, M., and Gajdusek, D.C. Infection of a new simian foamy virus from a spider monkey brain culture. J. Infec. Immun. 8: 804-813, 1973.

Johnson, R.T., and Gibbs, C.J., Jr. Koch's postulate and slow infections of the nervous system. Arch. Neurol. 30: 36-38, 1974.

Kirk, R.L., McDermid, E.M., Blake, N.M., Gajdusek, D.C., Leyshon, W.C., and MacLennan, R. Blood group serum protein and red cell enzyme groups of three Amerindian populations in Colombia. Am. J. Phys. Anthrop., in press.

Lewis, M.A., Frye, L.D., Gibbs, C.J., Jr., Chou, S.M., Cutchins, E.C., and Gajdusek, D.C. Isolation and characterization of two new unrelated herpesviruses from capuchin monkeys. J. Infec. Immun., in press.

Masters, C.L., Kakulas, B.A., Alpers, M.P., Gajdusek, D.C., and Gibbs, C.J., Jr. Experimental kuru and Creutzfeldt-Jakob disease in the gibbon (Hylobates lar), ape and Old World monkeys. J. Primatol., in press.

Mbagintao, I. Les esprits guerisseurs chez les dunkwi anga (Medicine spirits of the Dunkwi Anga. J. Soc. des Oceanistes 28: 337-343, 1972.

Peterson, D.A., Wolfe, L.G., Deinhardt, F., Gajdusek, D.C., and Gibbs, C.J., Jr. Transmission of kuru and Creutzfeldt-Jakob disease to marmoset monkeys. Intervirology 2: 14-20, 1973-74.

Plato, C., Brown, H.A., and Gajdusek, D.C. The dermatoglyphics of the Elema people from the Gulf District of Papua New Guinea. Am. J. Phys. Anthrop., in press.

Plato, C.C., Gajdusek, D.C., and MacLennan, R. The dermatoglyphics of the peoples of New Guinea: a review. In Mavalwala, J. (ed.): Dermatoglyphics, an International Perspective. The Hague, Mouton Publishers, in press.

Plato, C.C., Garruto, R.M., Hoff, C., and Baker, P.T. Digital and palmar dermatoglyphic patterns among southern Peruvian Quechua. Hum. Biol., in press.

Simmons, R.T., Graydon, J.J., Rodrigue, R.B., and Gajdusek, D.C. Blood group genetic data from the Western Highlands District, New Guinea, the adjacent Southern Highlands District and Western District, Papua. Am. J. Phys. Anthrop., in press.

Traub, R., Gajdusek, D.C., and Gibbs, C.J., Jr. Transmissible virus dementia. The relation of transmissible spongiform encephalopathy to Creutzfeldt-Jakob disease. In Kinsbourne, M., and Smith, L. (eds.): Aging, Dementia and Cerebral Function. Springfield, Illinois, Charles Thomas, Publishers, in press.

Traub, R.D., Gajdusek, D.C., and Gibbs, C.J., Jr. Suggested procedures in the conduct of biopsies and autopsies on patients with presenile dementia. J. Neurosurg., in press.

Wallace, G.D., Zigas, V., and Gajdusek, D.C. Toxoplasmosis and cats in New Guinea. Am. J. Trop. Med. Hyg. 23: 8-14, 1974.

Yu, R.K., Ledeen, R.W., Gajdusek, D.C., and Gibbs, C.J., Jr. Ganglioside changes in slow virus diseases: analyses of chimpanzee brain infected with kuru and Creutzfeldt-Jakob agents. Brain Res. 70: 103-112, 1974.

ANNUAL REPORT

July 1, 1973 through June 30, 1974
Branch of Electroencephalography and Clinical Neurophysiology
Intramural Research
National Institute of Neurological Diseases and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This Branch has continued to provide a clinical-diagnostic service for all in- and out-patients of the Clinical Center referred for electroencephalographic examination. Such referrals are either part of the routine diagnostic work-up or are part of specific research projects originating outside this Branch or from other Institutes.

In the twelve months since the time the last Report was prepared (April 13, 1973) about 1300 EEG tracings have been obtained and interpreted in our Laboratory, on the basis of corresponding referrals from the various Institutes, and specifically:

INSTITUTE	NO.	%
NINDS (OP 360)	861	66.3
NCI	174	13.4
NIMH	128	9.9
NHI	59	4.6
NIAMD	36	2.8
NIAID	35	2.7
NIDR/ehs	4	0.3
	1,297	100.0

This total has remained more or less stationary in the recent years. The largest number of referrals have continued to originate from NINDS and, predominantly, from the Branch of Surgical Neurology. All other Institutes combined, as in the recent past, have accounted for one third of the referrals. The above figures include both routine examinations and various types of special studies, such as those carried out in patients

with chronically implanted electrodes. This Branch has also cooperated with that of Surgical Neurology, NINDS in providing direct cortical recording and extemporaneous interpretations in 11 patients, in the course of their surgical exposure of the brain for the treatment of seizure disorders.

This form of service activity represents a considerable portion of the over-all activity of the Branch, requiring about 5 of its 11 man-years (1.2 professional and 3.4 technical-secretarial).

2. Research Activity

Of the seven projects listed in the previous Annual Report, one has been completed and two have been partially completed in the present fiscal period. One has been discontinued and the others, which are still pending, are also included in this Report together with three new projects. All but one of the investigation projects in the field of clinical neurophysiology deal with experimental epilepsy and, specifically, with various aspects of the mechanisms which are at the basis of a local epileptogenic process artificially established in the neocortex or hippocampus of cats.

One of the completed projects has investigated the effects of elevated levels of topically applied K^+ on the electrical manifestations and on the oxidative metabolism of a focal hippocampal epileptogenic process. With K^+ concentrations of 12 meq/l, the focus manifests itself with the typical interictal discharges alternating with rhythmically organized episodes of ictal activity which are followed by periods of post-ictal depression. Such cycles of epileptiform patterns keep recurring with stereotyped regularity as long as the K^+ concentration is kept constant. With lower concentrations the ictal (seizure) episodes tend to decrease in incidence or fail to appear altogether whereas at K^+ concentrations of 16 meq/l, they markedly increase while the accompanying post-ictal depression becomes shorter and shorter. At still higher K^+ concentrations, individual ictal episodes can no longer be identified since the post-ictal phase has practically disappeared and the preparation appears to be in the condition of continuous seizure activity ("status epilepticus"). Changes in intracellular NADH fluorescence, which reflect changes in the rate of oxidative metabolism, were also monitored during these various phases of hippocampal epileptiform activity. With interictal and ictal activity the oxidation rate would transiently increase to resume its resting level after each isolated discharge or in the postictal depression period. However, at very high K^+ concentrations, the overall changes in the rate of oxidation in the transition from interictal to ictal activity would tend to be less prominent

than at lower K^+ concentration. Additional details, a brief discussion and possible interpretation. of these findings are provided in the description of the specific project.

Another related project investigates these same general phenomena (electrographic and metabolic) in the neocortex and represents an attempt to compare the extent of their occurrence at the site of the primary epileptogenic process and at a distance from it, at the site of its "projected" activity. The latter site corresponds to a contralateral cortical area, homologous to that in which the epileptogenic focus has been originally induced. The changes in oxidation rate observed at the primary cortical focus were not dissimilar from those described for the hippocampal focus, but in spite of good electrographic evidence of indirect participation of contralateral cortical structures in the epileptogenic process, no changes in oxidation could be detected in these secondarily activated structures. In this series of experiments, specifically sensitive micro-electrodes have also been used to record simultaneously extracellular K^+ changes. K^+ was found to increase transiently in coincidence with the interictal and ictal discharges at the primary site of the "mirror" focus. These same techniques have been applied in another project to investigate more systematically the K^+ changes and their relation to oxidative metabolism in intact neural tissue under several experimental conditions. Preliminary results have shown that, as a consequence of local electrical stimulation, the extracellular K^+ increases and these changes are closely related to the local oxidation rate changes. Similar relations are observed in the course of epileptiform activity.

Another project by a different investigator utilizes similar techniques for the investigation of cortical metabolic changes which take place during the so-called "cortical arousal" induced by high frequency electrical stimulation of the pontomesencephalic reticular formation in cat. Preliminary findings would seem to indicate that, coincident with the characteristic electrographic changes in cortical activity ("desynchronization"), NADH decreases and these changes appear to be of at least the same order as, and probably even greater than those observed in coincidence with epileptiform activity.

The last experimental project which is a continuation of the same one started in the preceding fiscal period, is also in the field of experimental epilepsy and deals with the alteration in the physiological functions of cortical neurons which are reversibly participating in the epileptogenic process. Specifically, it is an investigation of the development of the "paroxysmal depolarization shift" or PDS (i.e. the characteristic membrane change observed in "epileptic" neurons) in cells of the visual cortex during the local

microiontophoretic application of an epileptogenic agent. The experimental approach and methods are described in the specific project outline. This project is partially completed and the results are currently analyzed and organized for publication. It has been possible to show a reversible and repeatable series of progressive alterations in the properties of the physiological response of visual neurons as a discrete and nearby focus of epileptiform activity begins to develop, becomes fully established and eventually disappears. Two distinct patterns of effects seem to occur. One consists of a progressive enhancement of the cell's response to a preferre stimulus; the other is a totally new response and is equivalen to a "triggered" PDS. The former could take place indepndentl of the activity of the epileptic focus but such a response would be elicited only by stimuli specific to the normal receptive field of the cell in question. The PDS response, on the other hand, was always associated with an interictal population discharge from the focus and could be "triggered" by both preferred and non-preferred stimuli -though with different latencies- (as well as occurring spontaneously). These interesting findings obtained by means of a technically elegant (and difficult) experimental approach may help to understand the basic mechanisms for the development of epileptiform activity.

In the clinical field, the major project is a continuation of that initiated in the previous fiscal period and carried out in close collaboration with the Branch of Neurological Surgery, NINDS. This project, which has been partially completed (with two papers ready to be submitted for publication) deals with a detailed analysis of epileptic patients which have undergone surgical treatment for their seizure disorder. The completed portion of this study consists of a long range follow-up of the effects of cortical excision on seizure frequency and pattern as well as on psychiatric and socioeconomic adjustment in patients affected by "temporal lobe" epilepsy, with correlative analyses of pre- and post-operative clinical and electroencephalographic data. The study is based on 124 patients which have been followed for 3-15 years after surgery. The evaluation of the results of treatment in the field of seizure disorders is generally difficult, particularly for the large group of cases in which the treatment was neither a total success nor a total failure. In this study a scoring method was used which a) permits to evaluate clinical and EEG results, both separately and in association; b) provides both a global, overall, result and a chronological evaluation throughout each post-operative year of follow-up; c) presents the results in a quantitative form and permits a comparison with other similar studies. The findings are provided in detail in the specific project description. This study has clearly demonstrated that the cure and improvement rate in seizures has

been significantly higher with "total" than with "partial" resections; and this seems more related to removal of the medial structures of the temporal lobe than to the extent of its lateral removal. It has also shown that follow-up periods of up to 3 years have little value for the assessment of therapeutic results; on the other hand, the proper evaluation of both clinical and EEG findings in the first or first two post-operative years may prove of reasonable prognostic value. This material is going to be published as a chapter of a monograph on "Surgical Treatment of Epilepsy." A separate study which is part of the same general project, deals with epileptic patients in which the decision for the surgical treatment of their seizure disorder had been reached on the basis of chronically implanted electrodes. Each of these cases presented as a complex diagnostic problem for the lateralization and localization of the epileptogenic process. The findings and results in these patients have been compared with those of a group of similarly complex cases in which the surgical excision had been determined on the basis of routine, scalp EEG procedures.

3. Other Activity

The Chief of the Branch is actively involved in editorial duties in several specialized national and international journals and is currently co-editor of the Handbook of Electroencephalography and Clinical Neurophysiology consisting of about 30 volumes. As past-President and member of the executive of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology, he is also actively involved in the organization and scientific program of the IX International Congress to be held in Amsterdam (The Netherlands) in 1977.

4. Programs considered for the near future.

The projects which are still pending should be completed in the coming fiscal period. The main field of interest and investigations of this Branch remains focused on the various aspects (both experimental and clinical) of seizure disorders. A close cooperation with the Branch of Surgical Neurology is again anticipated, especially in relation to the two new investigations (chronic cerebellar stimulation and section of Forel's field) which are planned by that Branch toward the treatment of epilepsy.

Serial No. NDS(I)-72 EEG/CN 1939a
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Fluorometric monitoring of intracellular redox changes in the intact cat brain

Previous Serial Number: Same

Principal Investigator: D. V. Lewis, M.D.

Other Investigators: M. J. O'Connor, M.D.

Cooperating Units: Division of Neurosurgery, Hospital of University of Pennsylvania

Man Years:

Total:	0.3
Professional:	0.2 (DVL)
Other:	0.1

Project Description:

Objectives: To study energy metabolism in the normal and penicillin-treated cat hippocampus and the effect of K^+ and electrical stimulation on this metabolic activity.

Methods employed: Intracellular redox changes as indicated by changes in the ratio of oxidized to reduced nicotine adenine dinucleotide (NAD) are monitored fluorometrically in vivo in the superficial 1 mm of the exposed hippocampus. Electrical activity in this same area is monitored with surface electrodes. The hippocampus is continuously bathed in Elliott's B_+ solution which is dripped onto the surface. Penicillin and/or K^+ are added to this solution and their effects on electrical and metabolic activity are recorded.

Major findings: The previous findings have been published and were essentially those stated in the previous report. In addition it was noted that progressive increases in oxidation rate were noted prior to each seizure as interictal activity increased and that the maximum oxidation rate was reached at the termination of each seizure.

Significance to Bio-medical Research and the Program of the Institute: These findings suggested that increased extracellular K^+ concentration significantly increased epileptiform activity and that onset and termination of ictal episodes showed characteristic changes in oxidative metabolism suggesting that active metabolic processes might play a role in the onset and termination of seizures.

Proposed Course of the Project: This project may be considered completed.

Honors and Awards: None.

Publications:

O'Connor, M. and Lewis, D.: Recurrent seizures induced by potassium in the penicillin treated hippocampus. Electroenceph. clin. Neurophysiol. 36: 337-345, 1974.

Lewis, D., O'Connor, M. and Schuette, W.: Oxidative metabolism during recurrent seizures in the penicillin treated hippocampus. Electroenceph. clin. Neurophysiol. 36 : 347-356, 1974.

Serial No. NDS(I)-72 EEG/CN 1939b
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Fluorometric monitoring of intracellular redox changes in human cerebral cortex

Previous Serial Number: Same

Principal Investigator: D. V. Lewis, M.D.

Other Investigator: W. Schutte, W. Whitehouse, J. B. Van Buren, M.D., Ph.D.

Cooperating Units: Surgical Neurology Branch, IR, NINDS, Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.15
Professional:	0.05 (DVL)
Other:	0.10

Project Description:

Objectives: Use of the television fluorometry system previously developed to study differences in oxidative response to electrical stimuli in normal and abnormal human cortex.

Methods employed: Using the previously described television system, fluorescence changes are recorded on video tape over a wide area of brain which has been exposed in the course of neurosurgical procedures. Energy metabolism is evaluated through analysis of transients both spontaneously occurring and induced by electrical stimulation.

Major findings: In addition to the previous findings this rate of oxidation induced in human cortex by electrical stimulation has been found to be proportionate to the stimulus intensity. Metabolic changes are localized within 1-2 cm of the stimulated area. No increases in oxidation rate have been observed during spontaneous interictal activity in the human cortex.

Significance to Bio-medical Research and the Program of the Institute: The findings obtained up to date would seem to indicate that epileptiform activity cannot be localized in human cortex by means of local changes in oxidative metabolism; however, this technique still enables one to study the kinetics of oxidative metabolism (e.g. stimulus response relationships using electrical stimuli and the oxidative response) in normal and epileptogenic cortical areas.

Proposed Course of the Project: More detailed analysis of stimulus response characteristics of normal cortex vs. cortex giving rise to epileptiform potentials. The progress of this project is dependent upon the availability of suitable patient material.

Honors and Awards: None.

Publications: Schutte, W., Lewis, D., O'Connor, M., Whitehouse, W., Van Buren, J.: Television fluorometer for monitoring oxidative metabolism. J. Assoc. Adv. Med. Instr. (To be published).

Serial No. NDS(I)-72 EEG/CN 1940(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Surgery of temporal lobe epilepsy. Pre- and postoperative EEG and clinical data

Previous Serial Number: Same (note slight modification of title and authorship)

Principal Investigator: C. Ajmone Marsan, M.D. and J. Van Buren, M.D., Ph.D.

Other Investigators: N. Mutsuga, M.D. and D. Sadowsky

Cooperating Units: Surgical Neurology Branch, IR, NINDS, Office of Biometry, NINDS

Man Years:

Total:	0.9
Professional:	0.7 (CAM)
Other:	0.2

Project Description:

Objectives: To analyze and correlate the characteristic features of preoperative EEG and clinical data, the site and extent of surgical removal and the clinical and EEG postoperative results.

Methods employed: This study is based on 143 patients which underwent surgical treatment for the relief of temporal lobe seizures. A number of pre- and postoperative data (EEG findings, seizure pattern(s), auras, psychological disorders, anamnestic data, etc.) are analyzed in the 124 cases which could be followed for 4 years or longer after surgery. Additional cases in which the preoperative studies induced the use of chronically implanted electrodes have been the object of a special investigation.

Major findings: The anamnestic data provided little hint as to the origin of the temporal lobe seizures, except for history of febrile convulsions and head trauma (with unconsciousness and/or skull fracture) which appeared, respectively, in about a quarter of the patients. The age of onset of seizures was about equally divided between the intervals below and above 10 years

of age. There was no significant difference in the length of seizure duration between patients with independent bitemporal and those with clearly unilateral epileptiform EEG activity; actually the average duration was slightly longer in the latter group. The most common auras were related to trunkal sensation (epigastric, cephalic, oropharyngeal). An aura was not reported by 10% of the patients. Typical "psychomotor" seizures (i.e. automatisms) were also lacking in somewhat less than one-third of the patients. Forty percent of the patients had significant personality and/or psychiatric problems with poor emotional control being the most common symptom. This was associated with mental retardation in 10% of the patients. An attempt has been made to evaluate and present both clinical and electrographic results of surgical treatment in a quantitative form and in relation to the duration of the postoperative follow-up period. The overall results showed 21% total abolition of seizures, 46% considerable improvement and 33% unchanged. Mortality and serious morbidity were less than 2%. These results can be compared with those reported in other studies. The cure and improvement rate in seizures was significantly higher with "total" vs. "partial" resection (e.g. cure rate of 32% vs. 4%), and this seemed more related to removal of the medial structures of the temporal lobe than to the extent of the lateral removal. Although following surgical treatment about one-third of the patients with personality or psychiatric abnormality showed alleviation of their symptoms, about one-fifth of the patients in whom symptoms of this type were not recorded preoperatively had note of them in the postoperative follow-up. Following surgical treatment, improvement in work capacity occurred in 50% of the cases, no change in 36% and deterioration in 12%. No differences were noted between the "total" and "partial" resection groups, suggesting that factors other than solely seizure control played a part. The combination of temporal and extratemporal or bitemporal epileptiform EEG abnormalities with unilateral preponderance did not appear to offer a poorer prognosis for seizure control with temporal resection than did the pure unitemporal foci. Twenty-one cases in the series showed postoperatively some degree of seizure reappearance, after a few to many years of freedom from seizures. Thus, short follow-up intervals (3 yrs or less) have little value in the study of seizure therapy. A minimum of 5 years would seem to be necessary for a more accurate assessment of surgical results. On the other hand, combining the postoperative clinical seizure rate and the EEG findings at the first or first two years of follow-up may prove of reasonable prognostic value. Thus, absence of seizures for 2 years with negative EEG's indicates a probably eventual cure rate of 72.5% which decreases to 30% if the EEG's remain positive. Similarly, the presence of early post-hospitalization seizures and positive EEG's indicate the 75% likelihood of total

failure which becomes a 50% failure rate if the EEG's are negative.

In a separate study, 17 cases presenting a complex diagnostic problem for the lateralization and localization of the epileptogenic process and in which depth and surface electrodes have been implanted and left in place for 1-2 weeks, have been analyzed and compared with a similar group of cases in which the surgical excision had been determined on the basis of routine scalp EEG procedures.

Significance to Bio-medical Research and Programs of the Institute: This project provides useful information of diagnostic, prognostic and therapeutic significance for the surgical treatment of the epilepsy.

Proposed Course of the Project: The project is considered completed and a paper has been prepared for publication to be included in a special monograph on the surgical treatment of epilepsy.

Honors and Awards: None.

Publications: Van Buren, J. M., Ajmone Marsan, C., Mutsuga, N. and Sadowsky, D. The surgery of temporal lobe epilepsy. In Rasmussen, T., et al. (eds.): The Surgical Treatment of Epilepsy. Raven Press (In Press).

Van Buren, J.M., Ajmone Marsan, C. and Mutsuga, N.: Temporal lobe seizures with additional foci treated by resection. J. Neurosurgery (In Press).

Serial No. NDS(I)-73 EEG/CN-2011
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Topographical analysis of cortical redox changes surrounding epileptic foci

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 EEG/CN 2094.

Serial No. NDS(I)-73 EEG/CN 2012
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of abnormal neuronal responses during the evolution of an epileptic focus in feline visual cortex

Previous Serial Number: Same (note slight modification of title)

Principal Investigator: J. S. Ebersole, M.D. and R. A. Levine, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.3
Professional:	2.0
Other:	0.3

Project Description:

Objectives: To characterize the development of the abnormal paroxysmal depolarization shift (PDS) in visual cortex neurons during the induction of an epileptic focus in the surrounding brain.

Methods employed: Cats are anethetized (pentobarbital), paralyzed (gallamine) and artificially respired. Temperature, blood pressure and expiratory CO_2 are monitored and maintained in physiologic range. Eyes are refracted and vision corrected with lenses. Twin glass micro-pipettes are positioned in the visual cortex (area 17). One records extracellular electrical activity, and the other iontophoreses penicillin G to establish the epileptic focus. A silver ball electrode records the ECoG. Receptive fields of the visual neurons are characterized before initiation of the penicillin focus. Recurrent preferred and non-preferred projected stimuli were presented before, during the development, and during the subsequent decline of the induced focus.

Major findings: We have demonstrated a progressive alteration in the response properties of visual cortex neurons during the evolution of an epileptic focus in the surrounding brain.

Two distinct types of abnormal response became evident. One was an enhancement of a cell's previously elicitable response to a preferred stimulus. The other was an entirely new response, the extracellular PDS. The enhanced physiologic response (EPR) could be elicited independently of the activity of the epileptic focus, but only with stimuli specific to the normal receptive field of the cell in question. The PDS response, on the contrary, was only evoked in association with an interictal spike from the focus. It could be triggered by both preferred and non-preferred stimuli, as well as occur spontaneously. Direct afferent stimulation of the cell did not seem to be required.

Significance to Bio-medical Research and Programs of the Institute: It is our contention, that the EPR represents a primary alteration in the properties of an individual cell or its synaptic connections due to penicillin, while the PDS response is a secondary manifestation of the effects of penicillin, dependent upon the altered interactions among a population of similarly affected cells. These conclusions support in part both the theory that the PDS is a population dependent phenomenon and that the initial abnormality is probably in individual cells.

Proposed Course of the Project: Use the same basic techniques to investigate further the initial cell abnormality (the EPR) and its relationship to developed epileptic activity (the PDS).

Honors and Awards: None.

Publications: Ebersole, J. S. and Levine, R. A.: Development of abnormal neuronal responses during the evolution of an epileptic focus in feline visual cortex (presented at the American Academy of Neurology meeting in San Francisco on April 26, 1974).

Serial No. NDS(I)-73 EEG/CN 2013
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The effects of epileptogenic agents upon the single unit responses in the cerebellar cortex

Previous Serial Number: Same

Principal Investigator: R. A. Levine, M.D.

Other Investigators: J. S. Ebersole, M.D.

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: Epileptiform activity of the type observed in elements of the cerebral cortex and other brain structures has never been reported following the application of topical agents to the surface of the cerebellum. Likewise, the deafferented cerebellum has not shown epileptiform activity to systemic agents. The present study will examine the effect of representative epileptogenic agents upon single unit responses of the adult cat's cerebellar cortex.

Methods employed: None.

Major findings: Nothing.

Proposed Course of the Project: This project has been terminated.

Honors and Wards: None.

Publications: None.

Serial No. NDS(I)-73 EEG/CN 2014(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical seizure patterns in patients with occipital epileptogenic foci

Previous Serial Number: Same.

Principal Investigator: C. Ajmone Marsan, M.D.

Other Investigators: None

Cooperating Units. None

Man Years:

Total:	0.00
Professional:	0.00
Other:	0.00

Project Description:

Objectives: To analyze the various sensory, motor autonomic and other symptoms which characterize the ictal episodes in patients with electrographic evidence of epileptiform activity in the occipital and para-occipital regions.

Methods employed: Data derived from about 50 patients with epileptic seizures selected on the basis of their EEG findings as specified above. All their records and clinical charts are reviewed and all the observed and described seizures for each subject are analyzed. The pattern(s) of each seizures and/or the various motor, sensory and other components of each ictal episode are correlated with the EEG localization and lateralization as well as with pathological, X-ray and other clinical findings.

Major findings: This project is still pending. No progress to be reported.

Significance to Bio-medical Research and Programs of the Institute: The understanding and treatment of epileptic disorders are among the major goals of this Institute. This project is complementary with a similar one (NDS(I)-71 EEG/CN 1868) which dealt with the seizure patterns of epileptic patients with

foci on different cortical regions and was completed last year. It is expected to provide useful information on the pathophysiology of this relatively uncommon localization as well as having practical diagnostic significance and, indirectly, helping in its surgical elimination.

Proposed Course of the Project: The collection of data will continue and their analysis should be completed within one year.

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-74 EEG/CN 2094
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Use of a television fluorometer to study stimultaneous oxidative and K^+ changes in primary and dependant mirror epileptogenic foci.

Previous Serial Number: None (incorporating part of Project No. 2011)

Principal Investigator: D. V. Lewis, M.D.

Other Investigators: W. Schutte, W. Whitehouse

Cooperating Units: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.8
Professional:	0.4 (DVL)
Other:	0.4

Project Description:

Objectives: To semiquantitatively study the changes in oxidation rate accompanying interictal and ictal activity in both a primary cortical epileptogenic focus (penicillin induced) and the contralateral cortical focus of projected (mirror) activity. Possibly to study as well oxidative changes with generalized epileptiform discharges.

Methods employed: The previously described television fluorometer records fluorescent images of the dorsal surfaces of the two cerebral hemispheres. A penicillin focus of epileptiform activity is established in a hemisphere and bilateral ECoG leads monitor this activity from the primary focus as well as any contralateral homologous (projected) activity. Simultaneous oxidative (NADH fluorescence) changes are studied in the primary and projected foci. As often as possible K^+ sensitive microelectrodes are used stimultaneously to record extracellular K^+ changes as well. The data are stored on video tape and analyzed at a later time.

Major findings: Consistent transient increases in both oxidation rate and extracellular K^+ accompanying interictal spike activity in the primary focus. Projected epileptiform sharp waves are seen in the contralateral homologous area of about half the voltage of the primary spikes, however no increase in either oxidation rate or extracellular K^+ has yet been observed with the projected sharp waves, even using signal averaging techniques.

Significance of Bio-medical Research and Programs of the Institute: Primary and projected discharges have very different effects on oxidative metabolism which might help elucidate the significance of projected epileptiform activity in human patients and possibly to differentiate in the operating room primary from projected activity.

Proposed Course of the Project: Obtain more data on the above and extend the project to generalized discharges elicited subcortically. Completion of this investigation study is expected during the coming fiscal period.

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-74 EEG/CN 2095
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Simultaneous monitoring of changes in extracellular K^+ concentration and oxidative metabolism in intact cat hippocampus and cerebral cortex

Previous Serial Number: None

Principal Investigator: D. V. Lewis, M.D.

Other Investigators: W. Schuette

Cooperating Units: Biomedical Engineering Branch of Div. of Research Services, Television Engineering Section of Clinical Center

Man Years:

Total:	0.50
Professional:	0.25 (DVL)
Other:	0.25

Project Description

Objectives: To study the relationship between active Na^+-K^+ transport and oxidative metabolism in intact neural tissues during electrical stimulation, evoked responses, and epileptiform activity.

Methods employed: Dual glass microelectrodes have been constructed using a K^+ exchange resin which can be used to monitor continuously extracellular K^+ concentration. These are inserted into the hippocampal (or cerebral) cortex in the area in which oxidative metabolism is being monitored fluorometrically (as described in Project No. 1939). The area is then stimulated electrically or exposed to epileptogenic agents, e.g. penicillin.

Major findings: Increases in extracellular K^+ and increases in oxidation rate, both secondary to electrical stimulation, are closely related. The total increase in extracellular K^+ secondary to a short (2-sec) burst of electrical pulses is linearly related to the total increase in oxygen uptake in the excited tissue. Kinetic analysis of the decline in extracellular K^+ following

stimuli agrees with the kinetics of the active Na^+-K^+ pump demonstrated in simple invertrebrate nervous tissue. Repetitive stimulation accelerates K^+ clearance from the extracellular space as well as increasing oxidation rate. Seizure activity is probably associated with a similar accelerated K^+ clearance.

Significance of Bio-medical Research and Programs of the Institute: The relation of active ion transport to oxidative metabolism in physiological and pathological (epilepsy) states might elucidate the mechanism of onset or termination of seizure activity.

Proposed Course of the Project: Continued study of energy metabolism and ionic fluxes in physiological (evoked responses) and pathological seizures) conditions. Project to be completed in the coming fiscal period.

Honors and Awards: None.

Publications: None.

Serial No. NDS(I)-74 EEG/CN 2096
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of electrical stimulation of the ponto-mesencephalic reticular formation on the electrical and metabolic activity of cortical areas in the acute cat

Previous Serial Number: None.

Principal Investigator: B. Vern, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: To study the effects of high frequency electrical stimulation of the reticular activating system on 1) re-dox level (NAD/NADH) and 2) electrographic activity of exposed cortex.

Methods employed: Adult cats are held in a stereotaxic instrument while immobilized with flaxedil; wound areas are infiltrated with xylocaine; small doses of Na thiopental are given intraveneously to prevent complete arousal at rest; artificial respiration with 100% O_2 is maintained, as is constant body temperature (37-38° C); various cortical areas are exposed by craniotomy; a concentric bipolar stimulating electrode is stereotaxically placed in the pontomesencephalic reticular formation (RF); and EEG is continuously recorded using Ag/AgCl cotton wick DC electrodes. Changes in surface cortical oxidation state are measured with a dual-beam fluorometer which is sensitive to changes in NADH level. High frequency stimuli (300 Hz) are delivered to the RF at current levels sufficient to "desynchronize" the EEG from "spindle" or slow wave states.

Major findings: Results using the dual-beam "spot" fluorometer have indicated that rather large (5-20%),

longlasting (30-100 sec), decreases in cortical NADH level can be induced by RF stimuli which simultaneously "activate" the EEG. The extent of NADH oxidation seems dependent on the level of barbiturate anesthesia: supplemental doses of chlorpromazine maximize NADH levels while inactivating the EEG, but in most cases they also apparently maximize the NADH oxidation induced by RF stimulation even though concomitant changes in EEG are not necessarily evident in this state. Variable reflectance changes at the cortical surface have been a practical and theoretical problem, but recent results using the television fluorometer system suggest the possibility of normalizing these changes over larger cortical areas than those seen with the spot fluorometer.

Significance to Bio-medical Research and Programs of the Institute: Data acquired to date suggest that NADH oxidation induced in cat cortex by RF stimulation is of the same order of magnitude as that induced by experimental focal epilepsy; they also suggest that while barbiturates do raise the NADH level by inhibiting cortical activity (directly and/or indirectly), they might not be as effective in uncoupling oxidative phosphorylation as has been reported in certain in vitro studies. There may be a dissociation between cortical activation and NADH oxidation under certain levels of barbiturate narcosis.

Proposed Course of the Project: Further work will be done with the TV fluorometer, which allows for a permanent and continuous record of the entire cortical surface. Evoked EEG and NADH changes will be sought for in relation to various RF stimulation sites. The effect of atropine on dissociating cortical arousal from RF stimulation will be compared to its metabolic effects. Attempts will also be made to measure extracellular potassium changes in response to RF stimulation using K-sensitive microelectrodes.

Honor and Awards: None.

Publications: None.

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Developmental and Metabolic Neurology Branch
National Institute of Neurological Diseases and Stroke

Roscoe O. Brady, Chief

The momentum generated through the creation of the Branch last year has continued at an accelerated pace. Among a number of accomplishments, two major research breakthroughs were made since the last annual report. The first was the establishment of the effectiveness of enzyme replacement therapy in hereditary lipid storage diseases. We showed that the administration of purified glucocerebrosidase isolated from human placental tissue to patients with Gaucher's disease causes a dramatic reduction in the quantity of accumulated lipid in the liver of these patients. Furthermore, the elevated level of glucocerebroside in the erythrocytes of Gaucher patients returned to normal following a single injection of glucocerebrosidase. The erythrocyte glucocerebroside has remained at the lowered level for over four months in these individuals. These demonstrations coupled with our previous work on enzyme replacement in Fabry's disease constitute the first definitive evidence that patients with inherited metabolic disorders can be significantly aided by administration of exogenous enzymes.

The second breakthrough was the conclusive identification of a patient with an entirely new form of inherited disease which we demonstrated to be due to a deficiency of an obligatory biosynthetic enzyme. All of the previously known metabolic disorders which include lipid storage diseases, mucopolysaccharidoses, glycogenoses and related conditions are caused by deficiencies of hydrolytic, catabolic enzymes. We established that the enzymatic defect in the newly discovered disorder in a severely retarded infant was an inability to synthesize complex glycolipids known as gangliosides larger than hematoside (ceramide-glucose-galactose-N-acetylneuraminic acid; ganglioside G_{M3}). All of the major components of the family of gangliosides in normal human brain contain two additional molecules of hexose and one to three more molecules of N-acetylneuraminic acid. The only ganglioside in the brain of the propositus was G_{M3}, this was due to a deficiency of the synthetic enzyme which catalyzes the formation of the next larger ganglioside homolog (Tay-Sachs ganglioside) via Reaction 1:

1. Ceramide-glucose-galactose-N-acetylneuraminic acid +

   UDP-N-acetylgalactosamine $\xrightarrow[\text{transferase}]{\text{aminosugar}}$

   Ceramide-glucose-galactose-(N-acetylneuraminic acid)-N-acetyl-galactosamine + UDP

We have previously observed a metabolic lesion of this nature in cultured mouse and hamster cell lines which had been transformed by the tumorigenic DNA viruses Simian virus 40 or polyoma virus. However, the case described is the first demonstration of such a metabolic defect in humans. This finding provides the opening wedge into a potentially large number of unknown hereditary disorders involving many tissues and the central nervous system in particular.

Other work in the Branch related to lipid storage diseases has also progressed satisfactorily. We have had good success in purifying sphingomyelinase from human placenta and we expect to initiate replacement therapy trials with this enzyme in patients with Niemann-Pick disease in the forthcoming year.

Work on the metabolic effects of tumorigenic virus transformation of cells has also proceeded very satisfactorily. We have shown that cells transformed with Moloney sarcoma virus, a tumorigenic RNA virus, also show the same synthetic enzymatic defect as cells transformed with the tumorigenic DNA viruses cited earlier. Cells transformed with the Kirsten strain of murine sarcoma virus, another tumorigenic RNA virus, show a different metabolic lesion. These cells are deficient in a galactosyltransferase required for the conversion of Tay-Sachs ganglioside to the next larger homolog (Reaction 2):

2. Ceramide-glucose-galactose-(N-acetylneuraminic acid)-N-

acetylgalactosamine + UDP-galactose $\xrightarrow[\text{transferase}]{\text{galactosyl}}$

Ceramide-glucose-galactose-(N-acetylneuraminic acid)-N-

acetylgalactosamine-galactose + UDP

A similar enzymatic block has recently been observed by us in cells which are transformed by chemical carcinogens or by X-irradiation.

Additional research along this line in collaboration with investigators in LMB, IR, NINDS, has provided an experimental system in which changes in the morphology of cells and ganglioside formation can be induced by the addition of salts of short chain fatty acids to the tissue culture medium. This ability to cause the switching on of a ganglioside synthesizing enzyme under carefully controlled experimental conditions provides a long-sought system for investigating the regulation of membrane lipid synthesis and is a unique opportunity to examine the correlation between the composition of complex lipids of cell membranes and morphological features.

Our investigations of the myelin-specific glycoprotein which was discovered in our laboratory have been extended in several directions. We have found that the size of this novel myelin protein decreases from a mass of 130,000 daltons to 110,000 daltons as myelin matures. We are actively investigating the nature of the 20,000 dalton fragment which is cleaved in

the course of normal myelination. We have evidence that this process is essential for the maturation of myelin since the residual myelin in myelin-deficient Quaking mice contains the higher molecular weight glycoprotein and compaction of myelin is impaired in these animals. Jimpy mice with virtually no myelin have none of the myelin glycoprotein in their central nervous system. This finding provides substantial evidence of the uniqueness of the glycoprotein to the myelin sheath. Additional work is in progress on the nature and fate of the myelin glycoprotein in brain tissue obtained from patients with multiple sclerosis.

The Section on Clinical Investigations and Therapeutics was involved in our discovery of the salutary effect of glucocerebrosidase replacement in patients with Gaucher's disease. Other work carried out by this Section during 1973/74 included studies on the etiology and pathogenesis of progressive cerebral degeneration. A new, comprehensive classification for three novel types of patients with cerebral lipidoses was established on the basis of clinical, histochemical, biochemical and electron microscopic evaluation of 13 patients. In other studies dealing with patients with mucopolysaccharidosis, it was shown that Type V category of patients with mucopolysaccharidosis consists of two enzymatically distinct entities. Certain of these patients have a deficiency of α-L-iduronidase activity in their tissues whereas others have a normal amount of this enzyme. In another study of the detailed composition of mucopolysaccharides in the brain of patients with these storage disorders, the morbidity of the disease has been shown to vary with the type and amount of accumulating polysaccharide.

Investigations were undertaken dealing with the abnormal metabolism of copper in a hitherto lethal condition known as Menkes' kinky hair disease. It was demonstrated that only a small fraction of orally administered copper was absorbed from the gut in these patients compared with normals, but the absorbed copper was retained three times longer than in the controls. Various conjugates of copper were systematically investigated in order to overcome the block in copper absorption. It was found that copper conjugated with tri-sodium nitrilo acetate has definite promise for the therapy of this disorder.

Other studies carried out during the past year dealt with the levels of anticonvulsant drugs in body fluids and the metabolism of these substances. The ratio of phenobarbital in cerebrospinal fluid to serum is 1:1.1, primidone 1:2.2, and diphenylhydantoin 1:5.6. It was found that there is a significant difference in the turnover and catabolism of these compounds in children and adults. Another important observation was the finding of a renal threshold for primidone. The dose of the drug usually administered is much too large and the amount given per kg of body weight will have to be reduced.

It was shown that there is a marked increase in a particular protein in lesions of the brain characterized by proliferation of glial elements. This protein designated as "Band 15" on electrophoresis has been isolated and is being characterized. It appears to be the same as the fibrous glial protein described by Eng and co-workers.

Extensive clinical and pathological studies have been carried out on arhinencephaly in 53 brain specimens which were available for histological examination. This congenital brain malformation is characterized by a wide spectrum of severity ranging from simple absence of smell to agenesis of a rudimentary brain incompatible with survival. Three types of this disorder were defined and criteria for their diagnosis and classification were established. The teratogenetic period was narrowed to 4-6 weeks of gestation and the severity was linked to varying dosage of deleterious or genetic factors.

In a recently undertaken study dealing with the pathogenesis and prevention of cerebrovascular disease an important preliminary observation was made that 12 of 37 patients with stroke had excessive plasma lipid levels and 9 of these had an abnormality of plasma lipoproteins. Although these patients may represent some selection bias, further studies and the therapeutic modification of these abnormalities will be undertaken.

In summary, the combination of basic research expertise with clinical capability has continued to be a unique and profitable venture resulting in markedly accelerated progress in the understanding and therapy of hereditary disorders. Several important honors were received by the Branch during the past year, among which were the Gairdner Foundation International Award, the first National Lecturer Award of the American Society of Biological Chemists, and the Distinguished Service Award of the Department of Health, Education and Welfare.

Serial No. NDS(I)-60 DMNB/CIT 706(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical, Genetic, Biochemical and Pathological Studies of Progressive Cerebral Degenerations, Mental Retardation and Birth Defects; with Emphasis on Familial Occurrence.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Jan K. Steusing

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.3
Other:	0.2

Project Description:

Objectives: The majority of chronic neurological disorders affect patients from their early years of life and constitute one of the greatest medical and social problems of our times. According to conservative estimates for this country, over four million people are permanently handicapped by mental retardation (frequently of familial type), birth defects, epilepsy and metabolic (genetic) disorders affecting the nervous system. Our main objectives are: a) to identify those conditions with neurological involvement which have increased familial incidence; b) to separate these patients into probably hereditary and acquired categories; c) to study the pathogenesis and etiology of certain of these conditions; d) to devise special diagnostic tests and e) to institute therapeutic modifications in several groups of these diseases and to explore preventive measures.

Methods Employed:

A. Diagnostic
1. Detailed general medical, neurological, psychological and developmental studies.
2. Genetic (pedigree type), cytogenetic and biochemical-genetic studies.
3. Special biochemical assays of blood, CSF, urine and saliva which

permit general assessment of protein, carbohydrate, lipid, glycolipid and nucleoprotein metabolism as well as the state of immunological responses.

4. Employment of special procedures when indicated, such as pneumoencephalography and arteriography, and in special instances, brain biopsy.
5. Employment of special enzymatic assays using patients' white blood cells, plasma, fibroblast tissue cultures and, occasionally, biopsy of liver, gut or brain.

B. Therapeutic

1. Use of approved and investigative pharmacological agents employing modifications of the drugs, dosages and others.
2. Use of corticosteroids for suppressing inflammatory reaction or enhancement of enzymatic activity.
3. Use of plasma, whole blood or white blood cells (obtained by plasmapheresis), for intravenous infusions as means of supplying deficient enzymes.
4. Infusion of the deficient trace elements, especially copper in absorptive defect of kinky hair disease.
5. Identification, isolation, purification and intravenous infusion of the respective deficient enzymes in special categories of genetic disorders.

Patient Material: 15 inpatients (463 patient days) and 16 outpatient visits.

Major Findings:

1. Among the diseases characterized by excessive storage of lipids in the neurons, the group of disorders known as non-sphingomyelin cerebral lipidoses (usually identified by a dozen eponymic names) is the most common. The age of onset, the course of the disease and clinical manifestations are different in various families. Thus far the etiology and pathogenesis of these disorders is obscure and investigations are greatly handicapped by a lack of comprehensive classification. Utilizing multidisciplinary approach which included: neurological, ophthalmological, histochemical, chemical and electron microscopic studies in 13 patients, we arrived at what appears a satisfactory working classification and contributed to the ultrastructure of lesions in each of the three principal types of the condition.

CLASSIFICATION OF CEREBRAL LIPIDOSIS PAST INFANCY

Clinical-Histological Criteria	Electron Microscopical Criteria	Tentative Combined Classification
Type 1	Category A	Type 1A
	Category B	Type 1B
Type 2	Category C	Type 2
Type 3	Category D	Type 3

In Type 1A, the onset of symptoms was between 2 and 6 years of age, with rapidly progressive mental and neurological deterioration, protracted visual involvement and death within 3 to 6 years. Chief histological feature was a marked distension of neurons with lipid material throughout the gray matter. The principal electron microscopic characteristics consisted of uniform inclusions consisting of convoluted, frequently paired membranes.

In Type 1B the clinical and histological characteristics were similar to those in Type 1A, but electron microscopical features were different. The inclusions were pleomorphic but typically characterized by delicate membranes that were arranged compactly or circular. In addition, some inclusions resembled zebra bodies and membranous cytoplasmic bodies.

In Type 2, the onset of symptoms was generally between 7 to 10 years of age; the main features consisted of rapid, retinal blindness but relatively slow progression of mental and motor deficits, occurrence of infrequent epileptic attacks and survival for 5 to over 12 years after the onset of the disease. The histological features were characterized by a greater involvement of neurons in laminae 2, 4 and 6 of the cerebral cortex. In electron micrographs, the inclusions generally consisted of focal densities, which may be associated with short linear membrane segments, or with compact or more widely separated paired membranes, as in Type 1A; finger print patterns were also present.

In Type 3, the onset of symptoms was past 11 years of age; the major clinical features included severe myoclonus and choreoathetosis with only moderate mental deterioration; visual abnormalities were either mild or absent; spasticity and motor weakness were not a feature. Chief histological findings included a more severe involvement of the basal ganglia, cerebellum and brainstem than those of the cerebral cortex. The inclusions seen in the electron microscope had resemblance to lipofuscin granules.

2. Menkes disease (kinky hair disease) and hepatolenticular degeneration are genetic disorders of copper metabolism characterized by specific systemic and neurological abnormalities. We were presented with an unique opportunity to study at the same time four patients with Menkes disease and several patients with Wilson's disease. Prior to therapeutic trials, it was necessary to obtain more information about turnover studies and absorption of copper in the kinky hair disease, since this was not done before. Utilizing ^{67}Cu, we have studied the whole-body turnover of copper and its incorporation and retention in various organs of patients with KHD, heterozygous mother, patients with HLD and normal controls. The mean of total body biological half-life of ^{67}Cu in KHD was 91.2, heterozygote 51.4, normals 29 and HLD 100 days. The mean of calculated percent oral dose absorption of ^{67}Cu was 13.2 for KHD, 46.4 for heterozygote, 54 for HLD and 52 for normals. The mean 8 days' retention of ^{67}Cu in the liver in KHD was 74%, heterozygote 45%, and normals 42%. The mean of stools and urine excretion of ^{67}Cu in KHD was 3.7% of the dose, heterozygote 9.6%, HLD 3.2% and normals 25.1%. Copper incorporation into red cells in KHD and HLD was normal. In both KHD and HLD , there is a

marked reduction of plasma copper and impaired biosynthesis of ceruloplasmin. In KHD ceruloplasmin deficiency and low tissue copper are probably related to reduced absorption of copper while in HLD the absorption of copper is normal but probably not available at the proper liver sites or it cannot be utilized to form ceruloplasmin, resulting in excessive tissue storage of copper.

Simultaneously with the metabolic studies, we have attempted to overcome absorptive block by conjugating copper with various compounds; most of these proved of no help with the exception of NTA-Cu conjugate which has a notable promise. This study is in progress.

3. Fabry's disease is an X-linked genetically determined disorder which is characterized by excessive accumulation of trihexoside in body tissues. The affected males have shortened life-span; they suffer severe pain in their extremities and die usually of renal failure. We have evaluated clinical data on 71 patients with this disease, 18 of whom had also autopsy examination. The mean age of onset of symptoms was 9.8 ± 3.1 years which means that future therapeutic trials should begin early in the pediatric age group to prevent irreversible kidney damage. It was surprising that nearly half of the patients with the advanced disease had severe neurological involvement including cerebral infarctions. Coronary heart disease was also a common complication. Of the 71 patients, cutaneous angiokeratoma was present in 60, absent in 4 and not assessed in 7. Corneal opacities were nearly a constant finding but required slit-lamp examination. Of the 17 patients who died, 10 succumbed under 40 years of age, 5 under 50 and only 2 lived more than 51 years. All these patients had severe kidney damage, eight had coronary artery lesions with 3 myocardial infarctions and three had large cerebral infarctions. In collaboration with Dr. R. Brady's group, further plans to infuse a purified enzyme, trihexosidase, are in progress.

4. The overall incidence of congenital malformations of the central nervous system exceeds other body organs, accounting for 79% of all cases including multiple deformities. As a result of the great complexity of the CNS, the variety of malformations of this organ is extensive. The majority of the surviving patients with brain malformation have clinical features of cerebral palsy, epilepsy and various degrees of mental impairment. We have studied in detail clinical and neuropathological abnormalities in an entire spectrum of arhinencephalies in 53 patients. This led to the establishment of a comprehensive classification of arhinencephalies into four types: Type I encompassed 20 patients whose primary abnormality was absence of olfactory bulbs and tracts; this was associated with other mild malformations in a proportion of patients; Type II included 14 patients with absence of olfactory bulbs and tracts and pronounced malformations of the cerebrum and cerebellum but normal ventricular system; Type III consisted of 10 patients who, in addition to the absence of olfactory nerves and tracts, had also non-separated cerebral hemispheres with a single ventricle, their brains showed also various malformations of neuronal migration and hypoplasia; Type IV consisted of 9 patients whose brains differed from the patients of Type III by the presence of huge posterior cyst in communication with the single ventricle placed

anteriorly and limited posteriorly by fused hippocampi. Further studies will be needed to evaluate in detail abnormal morphogenesis during embryonic period and assess the environmental and genetic factors leading to these severe disorders of brain function.

Significance to Bio-Medical Research and the Program of the Institute: There are over one hundred crippling hereditary disorders and they affect usually multiple organs; however, either directly or indirectly the nervous system is involved in nearly all of them. The results presented here advance our knowledge in the genetic mechanisms of some of these disorders. Therapeutic modifications of the course of the disease and changes in metabolism are likely to provide us with better understanding of these disorders and their eventual management.

Proposed Course of the Project: Our efforts will continue in determinations of pathogenesis and etiology of individual genetic disorders. The considerable success in preliminary therapeutic trials in mucopolysaccharidosis, Menkes disease, Fabry's disease and Gaucher's disease would be extended using improved approaches as we learn more; similar attempts will be extended to other hereditary diseases.

Honors and Awards: Clinical Associate Professor of Neurology, George Washington University Medical School (no remuneration)

Publications:

Dekaban, A.S.: Brain Dysfunction in Congenital Malformations of the Nervous System. In: Gaull, G.E. (ed.) Biology of Brain Dysfunction (in press).

Serial No. NDS(1)-61 DMN/EG 815
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Metabolism of Complex Lipids of Nervous Tissue, Studies on Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease, Krabbe's Disease and Metachromatic Leukodystrophy.

Previous Serial Number: Same.

Principal Investigator: P. G. Pentchev, Ph.D.

Other Investigators: R. O. Brady, M.D., A. E. Gal, Ph.D., J. F. Tallman, Ph.D.; G. E. Mook and S. R. Hibbert.

Cooperating Units: Weizmann Institute of Science, Rehovot, Israel.

Man Years:

Total:	4.8
Professional:	2.3
Other:	2.5

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebrosides, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states, and (4) to provide diagnostic and therapeutic procedures for the amelioration and control of the lipid storage diseases.

Methods: Glucocerebroside and galactocerebroside labeled with ^{14}C in either the hexose or fatty acid portion of the molecule have been synthesized. ^{14}C-labeled sphingomyelin and gluco-and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetra hexoside (globoside) uniformly labeled with radioactive hydrogen-^{3}H have been prepared. The metabolism of these labeled materials has been investigated *in vivo* and *in vitro*. Human placental tissue has been found to be a good source of sphingolipid hydrolases. Classical and newly developed methods of enzyme purification have been scaled up so that large quantities of placental tissue can be processed.

Major Findings:
(1) Glucocerebrosidase has been isolated in sufficient quantities to begin enzyme infusion studies in patients with Gaucher's disease.
(2) Glucocerebrosidase has been infused in two Gaucher patients with very promising results. There was a substantial and sustained reduction of the accumulated glucocerebroside in the liver and blood of the patients.
(3) A method has been developed whereby human placental sphingomyelinase can be isolated in homogeneous form.
(4) The "Sea-blue histiocyte" syndrome has been biochemically identified as a deficiency of sphingomyelinase and the disorder is therefore a modified form of Niemann-Pick disease.
(5) The isolation of α-galactosidase has been simplified so that purification of this enzyme can be scaled up for additional injections into patients with Fabry's disease.
(6) We continue to serve as a center for the diagnosis of patients and detection of carriers for all of the lipid storage diseases. Requests and samples come from all over the world for these assays. Much current work is devoted to the monitoring of pregnancies at risk for these heritable metabolic disorders. During the past year we monitored 32 cases and found nine affected individuals and ten carriers.

Significance: The results obtained from enzyme infusion in patients with Fabry's and Gaucher's disease are very encouraging. It seems very likely that enzyme replacement therapy will be of considerable benefit to patients with these diseases.

Proposed course: We will undertake an investigation of the long term effects of enzyme administration on the clinical course of Fabry's and Gaucher's disease. We will also investigate the effect of enzyme replacetherapy in patients with Niemann Pick disease now that we have obtained a completely purified preparation of the requisite sphingomyelinase.

Honors and Awards:

1. R. O. Brady, The Gairdner Foundation International Award, October 1973.
2. R. O. Brady, The Distinguished Service Award, Department of Health, Education and Welfare, April 1974.
3. R. O. Brady, National Lecturer Award, American Society of Biological Chemists, June 1974 (first recipient).

Publications:

1. Brady, R. O., The abnormal biochemistry of inherited disorders of lipid metabolism. Fed. Proc. 32: 1660-1667, 1973.

2. Brady, R. O., Hereditary fat-metabolism diseases. Sci. Am. 229: 88-97, 1973.

3. Brady, R. O., Inborn errors of lipid metabolism. In Meister, A. (Ed.): Advances in Enzymology Vol. 38, New York, John Wiley and Sons, 1973, pp. 293-315.

4. Pentchev, P. G., Brady, R. O., Hibbert, S. R., Gal, A. E. and Shapiro, D. Isolation and characterization of glucocerebrosidase from human placental tissue. J. Biol. Chem., 248: 5256-5261, 1973.

Serial No. NDS(1)-61 DMN/EG 816
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: Same.

Principal Investigator: J. F. Tallman, Ph.D.

Other Investigators: R. O. Brady, M.D., A. E. Gal, Ph.D.; J. M. Quirk, G. E. Mook.

Cooperating Units: None.

Man Years:

Total:	2.8
Professional:	1.3
Other:	1.5

Project Description:

Objectives: To investigate the normal relationship of human hexosaminidases A and B, their involvement in the metabolism of gangliosides and the derangement of these enzymes in patients with Tay-Sachs disease.

Methods: Highly purified β-hexosaminidase A and B have been prepared from human placental tissue using column chromatographic and ion exchange methods. The artificial fluorogenic substrate 4-methylumbelliferyl-β-N-acetylglucosaminide was used routinely to monitor enzyme activity. Tay-Sachs ganglioside N-acetylgalactosaminyl-(N-acetylneuraminosyl)-galactosyl-glucosylceramide was labelled in the N-acetylgalactosaminyl portion of the molecule with ^{14}C by an enzymatic synthetic scheme utilizing UDP-N-acetylgalactosamine-1-^{14}C. The asialo derivative of this compound was prepared by galactose oxidase treatment of N-acetylgalactosaminylgalactosyl glucosylceramide followed by reduction with tritiated sodium borohydride.

Major Findings: The complete purification of both forms of hexosaminidase has been accomplished. The enzymes have identical molecular weights and each is composed of four subunits of identical size. It is possible to convert hexosaminidase A into a "B-like" enzyme by heating it at pHs near neutrality. This "B-like" enzyme possessed identical electrophoretic mobility to the isolated hexosaminidase B and exhibited subunit interactions characteristics of the purified hexosaminidase B.

Both enzymes showed similar kinetic properties when artificial substrates were used to monitor activity. Hexosaminidase A and B possessed activity toward various lipidic substrates including the Tay-Sachs ganglioside in the presence of detergent.

Based on this examination of the properties of the purified enzymes, we have proposed that the two enzymes exist as conformational isomers of each other. A model for Tay-Sachs disease and the various variant forms was proposed based on the relative stability of these conformers and the prediction of a benign hexosaminidase A deficiency was made. This prediction has subsequently been confirmed.

Significance: These studies represent the beginning of an understanding of the molecular pathology of this group of the sphingolipidoses. Based on these findings, we now may consider rational therapeutic measures for alleviating this disorder and assisting in the prenatal diagnosis of the disease. We have continued with a moderate size program for the detection of carriers and patients with Tay-Sachs disease.

Proposed Course: We shall extend our work on the interactions between the purified enzyme conformers and the interconversion of A to B. The kinetic and physical properties of the abnormal enzyme from Tay-Sachs patients will be studied and this information should provide for the complete understanding of the molecular pathology of Tay-Sachs disease.

Publications:

1. Tallman, J. F., Pentchev, P. G. and Brady, R. O. An enzymological approach to the sphingolipidoses. Enzyme, 1973, in press.

2. Tallman, J. F.: Preparation and use of labelled glycoconjugates of the sphingolipids In: Montreuil, J. (Ed.), Colloque du C.N.R.S. sur les Glycoconjuges, 1973.

3. Tallman, J. F., The enzymology of Tay-Sachs disease and its variant forms. In: Buchwald, N. (Ed.): Brain Mechanisms in Mental Retardation, UCLA Medical Forum, in press.

4. Tallman, J. F. and Brady, R. O.: In Rosenberg, A (Ed.): The Biological Role of the Sialic Acids. in press.

5. Tallman, J. F., Brady, R. O., Quirk, J. M., Villalba, M. and Gal, A. E.: Isolation and relationship of human hexosaminidases. J. Biol. Chem. In press.

Serial No. NDS(I)-63 DMNB/CIT 1026(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical and Biochemical Studies on Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Ken Fujitani, M.D. and George Constantopoulos, Ph.D.

Cooperating Units: None

Man Years:

Total: 1.2
Professional: 1.2
Other: -

Project Description:

Objectives: There are close to 2 million people in the U.S. who are subject to epileptic attacks due to various causes. The onset of seizures is during childhood in over 65 per cent of these patients. Idiopathic (cryptogenic) epilepsy is the most common. Thus, there is a great need for broad as well as specific studies in the field of epilepsy, especially at the time of its onset. In the first place, we wish to obtain a better understanding of the underlying mechanisms of epileptic seizures and improved therapeutic procedures; this eventually should be followed by suitable preventive measures. Our present specific approaches consist of employment of high fat diet for control of seizures during early childhood and of progesterone for catamenial exacerbation of seizures. Elucidation of the mechanism of action of these regimens will be carried out in both patients and in laboratory animals. In addition, we have originated studies on altered modes of metabolism of anticonvulsant drugs in different patients, especially depending on age and genetic constitution.

Patient Material: 29 inpatients (725 patient days) and 28 outpatient visits.

Methods Employed:
1. General medical, developmental and neurological examinations, including routine and special laboratory tests.
2. Modified electroencephalographic studies and special performance testing.
3. Pneumoencephalography and arteriography, when indicated.

4. Determination of partitioned lipids and amino acids as well as assays of carbohydrate and endocrinological function.
5. Application of special therapeutic procedures including ketogenic diet and use of certain hormones (progesterone, corticosteroids).
6. Determination of anticonvulsant drugs in serum, CSF and urine during modified metabolic states and while on experimentally defined different doses of medication.

Major Findings:

1. The clinical manifestations, the therapeutic results attained and final prognosis of epilepsy differ considerably in children and adults. It is now appreciated that serum levels of anticonvulsant drugs are better indicators for arriving at the optimal therapy in an individual patient than empirically derived tentative dosage. Our preliminary observation suggested that drug metabolism and the response of children to anticonvulsant medication is different than in adults. It was, therefore, felt important to study these differences in detail with a view of obtaining better therapeutic indicators for children and for adults.

Seven children with a mean age of 9 years (range 5-12) and six adults all suffering from frequent epileptic seizures were the subject of this study. The blood was drawn in a fasting state 14 hours after the last dose of medication, and on the same date 24-hour urine was collected. The patients were maintained on various combinations of phenobarbital (PB), diphenyl-hydantoin (DPH) and primidone (PRM) and the dosages were changed by 50 per cent stepwise three times each for duration of 10 days or more. The extraction of the drugs from serum and urine and subsequent determination of PB, DPH and PRM was done according to the method of Kupferberg (1970) with some modifications. On repetitive studies, the standard error of mean deviations did not exceed 2.5% for PB and PRM and 4% for DPH.

We have found that although mean doses of PB and PRM per kg body weight/day in children was higher by 40-60% than in adults, the childrens' plasma levels and urinary excretion of these drugs was lower by 30-50%. This indicates that the metabolism of these drugs in children is at least twice as fast as in adults, converting PRM to one of its major metabolites phenylethylmanomide and phenobarbital to p-hydroxyphenobarbital. Other findings of importance were considerable individual variation in plasma levels and urinary excretion of specific drugs, both in children and adults.

Of particular interest was the finding that the level of serum PRM tended to plateau at about 4-7 µg/ml even if the dose of PRM was increased from 10 to 35 mg/kg. This indicates that there must be renal threshold for blood concentration of PRM which finding should mitigate against using doses of PRM above 20 mg/kg/day.

The serum levels of DPH in children were even more strikingly lower than in adults on comparable dose per kg/day.

The urinary excretion of DPH was negligible, but most of it was metabolized and eliminated in the urine in a form of 5-(p-hydroxyphenyl)-5-phenylhydantoin. About one-third of PRM was excreted unchanged in the urine, one third converted to PB and one-third excreted as phenylethylmanomide.

The ratios of phenobarbital in CSF:serum was 1 : 1.1, of primidone it was 1 : 2.2 and of diphenylhydantoin it was 1 : 5.6.

2. The association of an increased severity and frequency of seizures prior to or during menses in female epileptic patients has been repeatedly observed and referred to as catamenial epilepsy. When the marked increase in the seizures around the menstrual period was associated with uncontrollable behavior which prevented school attendance, we have decided to use progesterone in a form of three intravenous injections at bi-weekly intervals of medroxy-progesterone 250 mg in order to arrest menstruation. This proved successful in virtually eliminating seizures and improving behavior during the 11 months when the menses were arrested. Recurrence of menstrual periods was again associated with an increase of epileptic activity which, in turn, improved after a second set of 3 depot injections of medroxyprogesterone.

Significance to Bio-Medical Research and the Program of the Institute: Our understanding of the pathogenesis of epileptic seizures and their control is still meager. Many young children become severely and permamently retarded as a result of frequent seizures. Biochemical approach to epilepsy is likely to provide us with better understanding of this syndrome complex and it may help us in the therapy of this distressing disorder. Present investigations enlarge the overall scope of the study on epilepsy which is one of the major research efforts of NINDS.

Proposed Course of the Project: The metabolic studies of the anti-convulsant drugs as well as various endocrinological and dietary (ketogenic) modifications of seizures will be continued.

Honors and Awards: None

Publications:

Fujitani, K., Dekaban, A.S. and Zimmerman, A.W.: Comparison of Serum Levels and Urinary Excretion of Three Major Anticonvulsants Between Children and Adults. Brain and Development (in press).

Zimmerman, A.W., Holden, K.R., Reiter, E.O. and Dekaban, A.S.: Medroxy-progesterone Acetate in the Treatment of Seizures Associated with Menstruation. J. Pediat. 83: 959-963, 1973.

Serial No. NDS(1)-66 DMN/EG 1309
1. Developmental and Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Metabolism of Sphingolipids in Tumor Tissues.

Previous Serial Number: Same.

Principal Investigator: P. H. Fishman, Ph.D.

Other Investigators: R. O. Brady, M.D., P. L. Coleman, Ph.D.; R. M. Bradley.

Cooperating Units: Macromolecular Biology Section, Laboratory of Cell Biology, National Cancer Institute
Viral Leukemia and Lymphoma Branch, National Cancer Institute
Molecular Biology Section, Viral Carcinogenesis Branch, National Cancer Institute
Laboratory of Molecular Biology, National Institute of Neurological Diseases and Stroke.

Man Years:

Total:	2.2
Professional:	1.7
Other:	0.5

Project Description:

Objectives: To determine the mechanism which underlies the alterations of glycosphingolipid biosynthesis in neoplastic tissues; to investigate the function of membrane glycosphingolipids in the regulation of cell proliferation and in their involvement in cell morphology. Also emphasis will be placed on abnormal glycosphingolipid biosynthesis in other pathological conditions.

Methods: The metabolism of glycosphingolipids is studied in normal contact-inhibited and tumorigenic transformed cells in tissue culture. Metabolism in vivo is determined by adding ^{14}C-or ^{3}H-labeled precursors to the culture medium and isolating the labeled glycosphingolipids. Catabolism is measured in vitro with specifically labeled (^{14}C and ^{3}H) glycosphingolipids prepared in our laboratory. Biosynthesis in vitro is determined by measuring the activities of the various glycosyltransferases involved in glycosphingolipid formation. Activities of surface glycosyltransferases similarly are detected on intact cells. Surface

oligosaccharides are analyzed by selective oxidation with galactose oxidase or periodate and subsequent reduction with NaB^3H_4 before and after treatment of the intact cells with neuraminidase.

Major Findings: Following infection of Swiss or Balb 3T3 mouse cells with a high titer of Moloney sarcoma virus (a tumorigenic RNA virus), there is a loss of gangliosides more complex than G_{M3} (sialyllactosyl ceramide). This is due to a rapid loss of UDP-N-acetylgalactosamine: G_{M3} N-acetylgalactosaminyltransferase activity which loss parallels the morphological transformation of the cells. This specific block in ganglioside biosynthesis is the same as that previously observed by our laboratory in oncogenic DNA virus-transformed mouse cells such as SV 40 and Polyoma virus. Other glycosyltransferase activities were not decreased and infection of cells with the non-transforming Moloney leukemia virus had no effect on ganglioside metabolism. Balb 3T3 cells transformed by another RNA virus, the Kirsten sarcoma virus, are missing gangliosides G_{M1} and G_{D1a} and accumulate G_{M2}. There is no detectable UDP-galactose: G_{M2} galactosyltransferase activity in these transformed cells. The same defect was observed in several other subclones transformed with this virus. Other glycosyltransferases involved in ganglioside biosynthesis are not affected. In contrast to cells transformed by the Moloney sarcoma virus, the Kirsten sarcoma virus-transformed cells are nonproducers; they do not release type C particles. When the same clone of normal Balb 3T3 cells is transformed with the chemical carcinogens methylcholanthrene or benzpyrene or by X-irradiation, there is also a deficiency in this galactosyltransferase and a reduction in gangliosides G_{M1} and G_{D1a}. These results suggest that various oncogenic agents can induce the same biochemical change in the same cell line. Kinetic analysis of the residual galactosyltransferase activity in chemically transformed cells and the residual N-acetylgalactosaminyltransferase in DNA virus-transformed mouse cells indicates that the properties of these enzymes are similar to the enzymes from normal cells. Thus, malignant transformation is most likely depressing the synthesis of these glycosyltransferases.

HeLa cells, a continuous human cell line derived from cervical carcinoma, undergo a morphological change when grown in the presence of butyrate. The cells become flatter, extend processes, and stop growing. Butyrate-treated cells have an increased content of ganglioside G_{M3} and higher levels of CMP-sialic acid: lactosylceramide sialytransferase activity (10 to 30 fold more than control cells). The increase in sialyltransferase activity parallels the shape changes and both events are blocked by cycloheximide and Actinomycin D. This is the first report of the induction of glycolipid biosynthesis. The effect of butyrate is specific as there is no induction of other glycosyltransferases and removal of the butyrate causes the cells to resume their normal shape and reassume a low sialyltransferase activity. This model system will permit us to investigate the regulation of glycosphingolipid biosynthesis and possible regulatory malfunctions resulting from malignant transformation. These findings also support the role of membrane glycosphingolipids in the morphology and growth of cells.

Cholera toxin inhibits DNA synthesis and growth in cultured cells by stimulating adenyl cyclase activity and elevating cyclic AMP levels. Cholera toxin binds in a highly specific fashion to ganglioside G_{M1}, its putative surface receptor. To further elucidate the interaction of toxin with gangliosides, the effects of cholera toxin were investigated on three transformed mouse cell lines varying in their G_{M1} content and UDP-galactose: G_{M2} galactosyltransferase activity. There is an excellent correlation between G_{M1} content and sensitivity to cholera toxin. Cells with the highest G_{M1} content bind the most ^{131}I-cholera toxin; stimulation of adenyl cylase and inhibition of DNA synthesis occurs at the lowest concentrations of cholera toxin (10^{-12}M). Cells with no detectable G_{M1} are 100-fold less sensitive to the toxin. These studies further support the involvement of membrane gangliosides in the control of cell proliferation and suggest that gangliosides may be receptors for other factors that regulate cell growth. Absence of these ganglioside receptors in tumor cells may explain their lack of growth control.

A patient with unusual lipid storage disease which is characterized by the accumulation of ganglioside G_{M3} in the patient's liver and brain has been identified. In contrast to the metabolic defects in the other sphingolipidoses, the accumulation of G_{M3} is not the result of a defective catabolic reaction, but is the first disorder caused by a deficiency in ganglioside biosynthesis to be described in man. No UDP-GalNAc:G_{M3} N-acetylgalactosaminyltransferase activity was detectable in the patient's tissues. Other ganglioside glycosyltransferase activities were present which indicated a specificity of the defect. Our knowledge of the functioning of gangliosides is quite limited; however, the far ranging and devastating effects of deficient ganglioside biosynthesis during development points out their important role in the development of the central nervous system and visceral organs.

Publications:

1. Brady, R. O.: Biochemical Correlates of Virus-transformed Cells. In Zeman, W., and Lennette, E. H. (Eds.): Slow Virus Diseases. Baltimore, Williams and Wilkins Co., 1974, pp. 130-141.

2. Fishman, P. H., Brady, R. O., Bradley, R. M., Aaronson, S. A., and Todaro, G. J.: Absence of a Specific Ganglioside Galactosyltransferase in Murine Sarcoma Virus Transformed Mouse Cells. Proc. Natl. Acad. Sci. USA 71: 298-301, 1974.

3. Brady, R. O., and Fishman, P. H.: Alterations in the Pattern and Synthesis of Gangliosides in Tumorigenic Virus-Transformed Cells. In Clarkson, B. and Baserga, R. (Eds.): Control of Proliferation in Animal Cells. Cold Spring Harbor, N. Y., The Cold Spring Harbor Laboratory, 1974, in press.

4. Brady, R. O., and Fishman, P. H.: Biosynthesis of Glycolipids in Virus-Transformed Cells. Biochim. Biophys. Acta, 1974, in press.

5. Brady, R. O., and Fishman, P. H.: Alterations of Complex Lipid Metabolism in Tumorigenic DNA and RNA Virus-Transformed Cell Lines. In Schultz, J. and Block, R. (Eds.): Membrane Transformations in Neoplasia. New York, Academic Press, 1974, in press.

6. Fishman, P. H., and Brady, R. O.: The Altered Metabolism of Sialic Acid Containing Compounds in Tumorigenic Virus-Transformed Cells. In Rosenberg, A. and Schengrund, C. L. (Eds.): Biological Roles of Sialic Acid. New York, Plenum Press, 1974, in press.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: Same.

Principal Investigator: A. E. Gal, Ph.D.

Other Investigators: F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	1.2
Professional:	0.4
Other:	0.8

Project Description:

Objectives: To prepare sphingolipids labeled with radioactive isotopes. These compounds are used for metabolic studies and as diagnostic tools in investigations related to hereditary lipid storage diseases.

Methods and Major Findings: A multitude of approaches were used in labelling glycolipids such as chemical synthesis, partial synthesis, minor synthetic modifications, functional group exchange and tritium gas exposure. These methods could be classified into two categories: specific and non-specific labelling. The ideal approach is the specific labelling which consist of the tagging of a complex molecule at a predetermined atom. Total synthesis is the best way to accomplish this but up to now only few sphingolipids have been synthesized. We synthesized sphingosine, psychosine and galactocerebroside specifically labelled by total synthesis. However, our main effort is directed toward methods which would allow specific labelling of atoms yet would not necessitate tedious syntheses. An interesting technique which we developed is called the functional group exchange. A chemical group such as an acetyl or carboxyl is split from a molecule and is replaced with a similar but radioactive one. With this approach we could prepare aminosugars even gangliosides. Using the approach - minor synthetic modification; we prepared asialo ganglioside, Tay Sachs ganglioside and ceramide trihexoside. In this approach oxidation and reduction of an alcohol group in the molecule with a radioactive reducing agent would reestablish the original lipid in radioactive form. The lipids used as starting material for this approach were isolated from human tissues.

Tritium gas exposure, a non-specific approach, was repeatedly used for labelling ceramide dihexoside, dihexoside and globoside. By this method all the non-labile hydrogen atoms in a molecule become radioactive. This procedure is relatively simple but the purification of the resulting compounds are complex. Also this type of compound require more elaborate enzyme assays.

Significance: The compounds are indispensable in the detection, identification and isolation of enzymes connected to lipid storage diseases. Also studies related to qualitative and quantitative determination of enzymes in animal or human tissues necessitate these labelled substrates. Prenatal diagnoses are of rising importance. These labelled compounds play a key role in these diagnostic procedures. As a therapeutic approach, this branch initiated replacement therapy by the administration of the missing enzyme in hereditary diseases. The monitoring of the enzyme levels during and after this therapeutic procedure was done by the use of these radioactive substrates. It would be also of great interest to develop new methods which would allow to prepare relatively easily and inexpensively these compounds for the use of clinicians and for researchers who are not connected to large research centers.

Proposed Course: Many of the procedures for preparing radioactive substrates had to be developed in order to simplify the synthesis or preparation of these compounds and to make them more available. Other radioactive molecules which heretofore have not been labelled will be synthesized. The two new approaches will be studied further. One, based on the minor synthetic modification, will be extended to lipids which were previously labelled by more complex methods. The functional group exchange technique, which has a great future potential, will be refined and applied to numerous other molecules, particularly, to lipids which were labelled previously by conventional methods and to lipids which could not be labelled by any previously known methods.

Publications:

None.

Serial No. NDS(I)-68 DMNB/CIT 1523(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neurological, General Medical and Biochemical Aspects of Hurler, Hunter, Sanfilippo and Scheie Diseases: Basic Pathogenesis, Enzymology and Classification Criteria

Previous Serial Number: Same

Principal Investigators: Anatole S. Dekaban, M.D., Ph.D. and George Constantopoulos, Ph.D.

Other Investigators: Dennis F. Cain, Ph.D. and Marvin Zelkowitz, M.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.3
Other:	0.2

Project Description:

Objectives: The disorders of the metabolism of acid mucopolysaccharides are usually associated with severe dysfunction of the nervous system as well as liver, spleen, heart and others. Within the past ten years, several genetically different diseases were identified and our Unit provided significant contributions in delineating certain entities and pioneered in attempts at modifying the course of these diseases. The interest in these disorders is additionally increased by the fact that the derangement of the CNS relates to the excessive accumulation of lipid-staining substance in the neurons (leading eventually to their destruction), while liver, spleen, skin, kidneys and other organs store mucopolysaccharides. The pathogenesis and development of new diagnostic and enzymatic approaches will be investigated with a view to establish rational therapeutic trials.

Patient Material: 14 inpatients (398 patient days) and 17 outpatient visits.

Methods Employed:
1. General medical, developmental and neurological examinations including special tests as required.
2. Isolation, separation and determination of composition of various

mucopolysaccharides in urine, serum and cerebrospinal fluid.
3. Determination of the molecular weight distribution in the isolated mucopolysaccharides.
4. Establishment of skin fibroblast tissue cultures for the purpose of histochemical studies and chemical determination of AMPS.
5. Utilization of tissues from organs obtained by biopsy for determination of acid mucopolysaccharide composition and enzyme studies.
6. Extraction of tissue from organs obtained at autopsy for large scale recovery of the stored acid mucopolysaccharides and lipids.
7. Production of biologically labelled ^{35}S heparitin sulfate utilizing skin fibroblasts in the tissue culture from patients with Sanfilippo A variant. This labelled substrate will be then used for determination of respective enzymatic activity in leukocytes and plasma of patients and unaffected controls.

Major Findings: We have studied brains from seven of our patients who died with the diagnosis of mucopolysaccharidosis. Four of them were Type I (Hurler) one type IIIA (Sanfilippo) and one Type V (Scheie). The results of biochemical investigations can be summarized as follows: the content of mucopolysaccharides (AMPS) expressed as per cent of the fat free dry weight for mixed gray and white matter was: for Type I- 1.27 (mean), for Type II- 1.09, for Type IIIA- 0.9 and for Type V- o.45, whereas in normal control brain it was 0.33%. Partially degraded dermatan sulfate made up for most of the increases in AMPS content in Types I, II and V, while highly fragmented heparan sulfate was the principal AMPS in the brain of the patient with Type III. Heparan sulfate was also a sizable component in Type I and II.

Liver and brain of patients with Type I and one patient with Type V showed virtual absence of enzyme activity: phenyl-α-L-iduronidase. It appears, then that these patients until now classified into two different types must have much in common; further studies will be planned to investigate this particular aspect.

We have shown previously that except for certain patients with Type V mucopolysaccharidosis, all patients of Types I - IV had greatly increased AMPS in their cerebrospinal fluid; this was consistant with direct correlation with the absence or presence of mental deterioration. In subsequent study, we have used the chondroitinase ABC to hydrolyze AMPS in the cerebrospinal fluid of patients with Type I - III mucopolysaccharidosis. The non-hydrolyzed AMPS (predominantly heparan sulfate) accounted for 54% of total CSF mucopolysaccharides in Type I, for 55-62% for Type II and 82-100% in Type III. The Type III is characterized by predominant involvement of intellectual function and it appears probable that heparan sulfate is particularly toxic to neural elements; further studies of this aspect are planned.

Labelling of mucopolysaccharides in tissue culture with ^{35}S was accomplished by pulse-chase incubations with both Sanfilippo Type A and normal skin fibroblasts. These cells were compared as to total rate of incorporation

of ^{35}S/mg. protein and rates of incorporation into various AMPS fractions. Material was harvested, and, after protein digestion, separated by ion exchange chromatography. The 1.0 molar eluate was then recovered by ethanol precipitation. Mucopolysaccharide determination was then carried out by four different electrophoretic systems. By these parameters, we found that heparan sulfate accumulates only in the pulse-chased Sanfilippo fibroblasts. N-sulfate degradation revealed 80% of the label to be N-sulfated. Enzyme assays performed with both normal and pathological fibroblasts and leukocytes revealed that patients with the Type A Sanfilippo variant are unable to degrade the labelled substrate derived from Sanfilippo fibroblasts while normal cells demonstrated the ability to degrade this material. All cells assayed had O-sulfatase activity when tested against artificial substrate.

Significance to Bio-Medical Research and the Program of the Institute: Simultaneous application of basic and chemical studies to these generally sublethal disorders have provided promising data. The time may be not too distant when successful and practical modification of the course of the disease in various mucopolysaccharidoses will become possible. Also, the information obtained will be helpful in diagnosis and prevention of these devastating hereditiary disorders. Also, of considerable significance is proper classification of various types of mucopolysaccharidoses.

Proposed Course of the Project: This project carries promise and will be continued, while emphasis will be placed on enzymatic aspects of the problems concerned and on the basic interaction between disturbance of mucopolysaccharide and lipids. Therapeutic trials are also planned.

Honors and Awards: None

Publications:

Dekaban, A.S. and Herman, M.M.: Childhood,juvenile and adult cerebral lipidosis: are these different nosological entities? Arch. Path. 97: 65-73, 1974.

Serial No. NDS(I)-69 DMNB/CIT 1655
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of the Composition and Biosynthesis of Cerebral Proteins in Experimental Animals and Man

Previous Serial Number: Same

Principal Investigator: Dennis F. Cain, Ph.D.

Other Investigators: Anatole S. Dekaban, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: Brain proteins differ from those of other tissues particularly in the elevated levels of the acidic proteins. They are also rapidly synthesized and degraded - a phenomenon without a functional correlate.

An understanding of the role of these specialized aspects of protein metabolism in normal nervous tissue requires determination of the identity, composition and relative rates of synthesis and degradation, as well as the localization of individual proteins within various cell types and subcellular organelles. This information is particularly important in the case of the acidic proteins, some of which are known to be brain-specific. Acidic proteins in other tissues have been shown to exert a regulatory function in RNA and protein metabolism. The possibility of interaction of acidic proteins in nervous tissue with neurotransmitters and neuropharmacological agents is of especial significance in neural tissue.

The application of appropriate extraction and separation procedures will allow the determination of both the spectrum and the variability in the composition of the soluble proteins in normal human brain. Thus, changes in the proteins associated with neurological diseases may be examined. The purpose of such studies is to screen normal and pathological surgical and necropsy specimens for the presence or absence of particular protein fractions which may reflect

changes in cell structures and cell populations characteristic of various pathological states.

Methods Employed: Various protein fractions are extracted and purified by salt fractionation, isoelectric precipitation, gel filtration, ion exchange, chromatography, etc. These proteins are subsequently analyzed by electrophoresis and isoelectric focusing in a soluble acrylamide gel system. The specific purified protein fractions are also characterized by amino acid analysis, sedimentation and immunological procedures.

Evolving techniques for the separation of subcellular as well as whole cell units will continue to be evaluated for purposes of localization of the individual protein fractions. Tissues from specific areas of the brain and pathological specimens containing a predominant cell type will be especially valuable for this purpose.

Material: Brains and other organs from the patients obtained by biopsy or at autopsy are used.

Major Findings: Previously, we have demonstrated the presence of large amounts of two particular protein fractions (Band 15 and Band 20), and the marked decrease in another (Band 5) by electrophoretic separation of extracts of certain pathological brain biopsy specimens. One of these proteins (Band 15) has now been isolated in nearly pure form from two brain specimens by a combination of salt fractionation, dialysis and gel exclusion chromatography. The protein has a molecular weight in excess of one million as determined by gel filtration. On dissociation and reduction, it is converted to subunits of molecular weight of 45,000. The protein has an isoelectric point of 5.8. Analysis of sialic acid indicate about 1 mole of sialic per subunit. Amino acid analysis shows a high content (27-30%) of glutamic and aspartic acids.

We have prepared antiserum to the band 15 protein in rabbits. With the higher sensitivity of the antiserum, we have shown the protein to be absent in the other human organs examined. With the antibody, it has also been possible to demonstrate the presence of small amounts of this protein in normal brain tissue obtained during therapeutic surgery, whereas in some post-mortem brain specimens, we have not been able to demonstrate this protein, suggesting the possibility of isoelectric precipitation, aggregation, or autolysis. Using the antiserum, we have shown the presence of cross-reacting protein in the CSF of a patient months after surgery for glioma.

Significance to Bio-Medical Research and the Program of the Institute: There is some suggestive and still indirect evidence that specific cerebral proteins may be involved in the processes of memory and intellectual functions. Correlation between stimulatory events and the products of synthesis are lacking. There are large numbers of subjects with undifferentiated type of mental retardation who defy all investigative approaches in clarifying the underlying abnormality. Because of several technical difficulties, the brain

proteins have not been thus far explored in these and many types of other patients. The availability to us of surgical and post-mortem tissue of a number of hitherto obscure neurological diseases provides us with unique opportunity to study cerebral proteins in these patients.

Proposed Course of the Project: We will be unable to continue this project during FY 1975 because of shortage of staff and need to concentrate on direct clinical research of neurological disorders. The results obtained from this project are in press, and final report is being prepared.

Honors and Awards: None

Publications:

Cain, D.F., Ball, E.D. and Dekaban, A.S.: Proteins in Human Brain Tissue Obtained During Surgical Procedures. J. Neurochem. (1974, in press).

Serial No. NDS(1)-70 DMN/EG 1808
1. Developmental & Metabolic Neurology Branch
2. Enzymology and Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Glycoprotein Metabolism in the Myelinating Central Nervous System

Principal Investigator: R. H. Quarles, Ph.D.

Other Investigators: R. O. Brady, M.D., J. L. Everly, Ph.D., J.-M. Matthieu, M.D., A. Zimmerman, M.D., J. Poduslo, Ph.D.

Cooperating Units: Section on Cellular Neuropathology, LNNS, NINDS; Surgical Neurology Branch, NINDS

Man Years:

Total: 4.9
Professional: 4.9

Project Description:

Objectives: To investigate the biochemistry of cells of the nervous system with particular regard to glycoprotein components and their roles in myelination and demyelination. Other myelin and oligodendroglial proteins and lipids will also be examined with the ultimate objective of understanding the molecular mechanisms of myelin formation and breakdown. Emphasis will be placed on the major myelin-associated glycoprotein of the central nervous system and its role in demyelinating diseases such as multiple sclerosis.

Methods: Radioactive sugar precursors are used to label specifically central and peripheral nervous system glycoproteins. Myelin and other subcellular fractions are purified by differential centrifugation on sucrose gradients. Purified myelin is subfractioned into light, intermediate, and heavy fractions with different biochemical and morphological properties. Enzyme markers are used to characterize the different subcellular fractions. The membrane-bound proteins and glycoproteins are fractionated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Double label counting techniques are used for detecting the labelled glycoproteins on gels and revealing small differences between samples. Densitometric scanning of gels stained with Fast Green for proteins or periodic acid-Schiff reagent for glycoproteins is used for quantitation of individual protein components. Quantitation of individual lipids is carried out by thin layer chromatographic separation and

densitometric scanning of the TLC plates. Purification of the major myelin-associated glycoprotein involves solvent fractionation, preparative polyacrylamide gel electrophoresis, and column chromatographic techniques. Gas liquid chromatography and standard colorimetric procedures are used for quantitation of individual sugars in subcellular fractions and in purified glycoproteins.

Major Findings: Investigations have continued to center on the major myelin-associated glycoprotein of the mammalian CNS whose discovery by this laboratory was reported in last year's annual report. As measured by Schiff staining, the concentration of this glycoprotein per mg of total myelin protein remained nearly constant between 14 days and 60 days of age in the rat. During this period, the total amount of myelin recovered per brain increased more than 35 fold. Similar results were obtained in developmental studies on bovine and human brain. Although the concentration of the glycoprotein in myelin does not change much during development, double labelling experiments revealed that the glycoprotein synthesized between 12 and 18 days of age in the rat had a higher apparent molecular weight on SDS gels than that synthesized by rats which were 22 days of age or older. This difference in electrophoretic mobility probably reflects a difference in the carbohydrate moieties of the glycoprotein being synthesized at the different ages. This would result in a change in the oligosaccharide moieties on the surface of the myelin or oligodendroglial cell membranes. Consistent with this interpretation was the finding that there are developmental differences in the labelled glycopeptides derived from the glycoprotein peak by extensive pronase digestion. The glycopeptides have a simpler structure than the intact glycoprotein and are water soluble, and therefore should prove valuable in elucidating the exact chemical reason for the developmental difference. A similar developmental change in the newly synthesized glycoprotein was observed in the hamster and gerbil but could not be detected in the mouse. On the other hand, in the Quaking mutants of mice which fail to myelinate properly, the glycoprotein was of higher apparent molecular weight similar to that found in immature animals of other species. The consistent finding in all of these species was that in normal, mature myelin the glycoprotein was of the smaller molecular weight form.

Our studies on the Jimpy mutants of mice, which myelinate even less than the Quaking mutants, have been completed. Although we could isolate a pure myelin fraction from very immature normal mice with only traces of myelin, the fraction obtained from the Jimpy mice had none of the characteristics of classical myelin. We could not detect basic protein, proteolipid or galactocerebroside in the fraction, and electron microscopic examination revealed none of the typical lamellar structure of myelin. This fraction could be an extremely abnormal myelin, but more likely consists primarily of nonmyelin contaminants. Nevertheless, this study was extremely valuable for our studies on the glycoprotein of normal myelin, since the glycoprotein was absent in the fraction from Jimpy mice providing another very strong piece of evidence for the genuine association of the glycoprotein with myelin or myelin-related membranes in normal brains.

Our investigation of myelin subfractions in whole rat brain was completed and clearly revealed that a purified myelin fraction is biochemically and morphologically heterogeneous. The light fraction looked like mature, compact myelin in the electron microscope and had a high content of lipid and myelin-specific basic protein. In contrast, the heavy fraction contained smaller myelin fragments and single membranes, and appears to be enriched in loose myelin membranes and possibly in oligodendroglial plasma membranes. The glycoprotein and the myelin marker enzyme, 2'3' cyclic-AMP phosphohydrolase, were most abundant in the heavy fraction suggesting that they may be concentrated in the oligodendroglial plasma membrane and its region of transition to myelin. Since investigation of myelin subfractions appears to offer a very fruitful approach to understanding the structure and formation of myelin, we extended these studies to the developing brain and to specific regions of the mature CNS. In the adult rat CNS, most of the myelin from the brain stem and spinal cord was of the light variety, with very little intermediate myelin and small amounts of the heavy fraction. In contrast, the myelin from the cerebral hemispheres and subcortical white matter yielded substantial amounts of the intermediate fraction. In developing 16 day old rats the total yield of myelin was much reduced and consisted primarily of the light variety. This was somewhat surprising since we expected immature myelin to contain a higher proportion of uncompacted myelin and oligodendroglial plasma membranes similar to that which we observed in the heavy fraction from adult myelin. A possible explanation for the small amount of heavy myelin recovered from immature brain is that the very immature myelin has properties so different from mature myelin that it is not recovered in the myelin fraction at all. Indeed, metabolic experiments described in the last year's annual report provided evidence for the existence of such a membranous precursor of myelin in developing brains. Biochemical and electron-microscopic analyses of the regional and developmental subfractions are in progress in collaboration with Dr. Henry Webster of LNNS.

Since the studies on myelin subfractions have suggested that the glycoprotein is particularly concentrated in loose myelin and possibly on the surface of the myelin sheath and oligodendroglial cells, we have begun experiments involving techniques which are used for probing the molecules on the surfaces of cells. Plant lectins, such as Concanavalin A, bind specifically to particular carbohydrate residues. Dr. Amiram Daniel of the Neurosurgery Branch has provided us with radioactive lectins for use in these studies. We have shown that the radioactive Concanavalin binds to myelin and causes aggregation of myelin fragments. These effects are inhibited by the specific inhibitor of the lectin, α-methyglucoside. Also we have shown that myelin-associated glycoproteins bind to Concanavalin A linked to Sepharose, suggesting that they are the receptors for the lectin. Another procedure for labeling surface proteins is lactoperoxidase catalyzed iodination, and this technique was used in another laboratory to identify a high molecular weight protein on the surface of the myelin sheath. We used this technique together with fucose labelling to demonstrate that the major myelin-associated glycoprotein electrophoreses

very close to the iodinated surface protein but is distinct from it. This could indicate either that the glycoprotein is not exposed on the surface of myelin or that it is not effectively iodinated. Therefore, we are beginning experiments with galactose oxidase and [^{3}H]sodium borohydride reduction as a more suitable probe for surface glycoproteins.

Attempts to purify the major myelin-associated glycoprotein have continued. Since the glycoprotein accounts for approximately 1% of the myelin protein, only about a 100-fold purification from the isolated myelin is required to obtain the pure glycoprotein. We have achieved a 50-fold purification by a combination of solvent fractionation and preparative polyacrylamide gel electrophoresis. Therefore only about a two-fold additional purification is required to separate the glycoprotein from two nonglycoproteins which migrate with it during electrophoresis. We expect to have a procedure for the complete purification of the major myelin-associated glycoprotein within the next few months.

Since a major symptom of Menkes disease (insufficient copper transport in the gut) is a failure to properly myelinate, we have attempted to create a model of this disease by raising rats on a low copper diet. With appropriate treatment so that copper levels were reduced to sufficiently low levels, the affected animals formed significantly less myelin than controls. We are now doing biochemical studies to determine if the copper deficiency results in reduced amounts of normal myelin or if the myelin is biochemically abnormal.

Significance: The probable localization of the major myelin-associated glycoprotein in loose myelin membranes and in oligodendroglial plasma membranes, as suggested by the subfraction studies, has important implications for processes of myelination and demyelination. In this localization the glycoprotein would be accessible for interactions with other cells and with pathological agents such as antibodies and viruses.

With regard to myelin formation, there is considerable evidence in the literature indicating that cell surface glycoproteins are involved in cell-cell recognition phenomena and in specific interactions between cells. The myelin-associated glycoprotein could be involved in interactions between oligodendroglial and axonal membranes or between different layers of myelin membranes. The apparent developmental change in the carbohydrate structure of the major glycoprotein could be involved in these interactions. The finding that normal mature myelin always has the glycoprotein of smaller apparent molecular weight suggests that conversion to this form may be a prerequisite for proper compaction and maturation of myelin. Persistence of the glycoprotein of higher molecular weight as found in the Quaking mutants might prevent normal compaction of the myelin.

Many demyelinating diseases such as multiple sclerosis are believed to involve autoimmune and/or viral processes. Membrane glycoproteins are known to be cell surface antigens and receptors for viruses. Therefore, it is reasonable to suppose that the myelin-associated glycoprotein could be directly involved in demyelinating diseases. For example, in multiple

sclerosis a viral induced change in the sugars on the glycoprotein could cause it to be recognized as a foreign antigen and become an immunogenic substance.

Proposed Course: Once the procedure for the complete purification of myelin glycoprotein has been perfected, large amounts of the purified glycoprotein will be isolated for chemical characterization and for investigating the roles of the glycoprotein in developmental and pathological processes. The amino acid and sugar composition of the glycoprotein will be determined. The intact glycoprotein and glycopeptides derived from it will be used to determine the structure of its carbohydrate moieties. It should be possible to determine the exact chemical reason for the developmental change in the glycoprotein and to look for chemical abnormalities in multiple sclerosis and other demyelinating disorders.

The purified glycoprotein will be used to raise antibodies and to see if these antibodies cause demyelination or prevent myelination. Also experiments will be undertaken to see if the demyelinating antibodies in the serum of EAE animals or in multiple sclerosis patients are directed against the glycoprotein. Antibodies to the glycoprotein will also be tagged with ferritin in an attempt to precisely localize the glycoprotein in the brain by electron microscopic techniques.

Finally a collaborative study will be undertaken with Dr. Henry Webster and Dr. Paul Reier of LNNS in an attempt to precisely correlate biochemical events in myelin formation with morphological studies. We will use micro-adaptions of the techniques used in our subfraction studies on myelin in whole brain, while they will do conventional electron microscopy and freeze fracture studies. We expect this investigation to provide biochemical and morphological correlations during myelin formation with a precision not previously achieved.

Publications:

1. Brady, R. O. and Quarles, R. H.: The enzymology of myelination. Mol. C. Bioch. 2: 23-29, 1973.

2. Everly, J. L., Brady, R. O. and Quarles, R. H.: Evidence that the major protein in rat sciatic nerve myelin is a glycoprotein. J. Neurochem. 21: 329-334, 1973.

3. Glasgow, M. S. and Quarles, R. H.: Appearance of $^{3}H_2O$ in rat tissues resulting from the metabolism of L-[^{3}H]fucose by intestinal bacteria. Biochim. Biophys. Acta 304: 586-589, 1973.

4. Matthieu, J.-M. and Quarles, R. H.: Quantitative scanning of glycoproteins on polyacrylamide gels stained with periodic acid-Schiff reagent. Anal. Biochem. 55: 313-316, 1973.

5. Matthieu, J.-M., Quarles, R. H., Brady, R. O., and Webster, H. deF.: Variation of proteins, enzyme markers and gangliosides in myelin subfractions, Biochim. Biophys. Acta 329: 305-317, 1973.

6. Max, S. R. and Quarles, R. H.: Quantitative thin layer chromatography of gangliosides. In Touchstone, J. (Ed.): Quantitative Thin-Layer Chromatography, New York, John Wiley and Sons, 1973, pp. 235-251.

7. Quarles, R. H., Everly, J. L. and Brady, R. O.: Evidence for the close association of a glycoprotein with myelin in rat brain, J. Neurochem. 21: 1177-1191, 1973.

8. Quarles, R. H., Everly, J. L. and Brady, R. O.: Myelin-associated glycoprotein: a developmental change, Brain Res. 58: 506-509, 1973.

9. Druse, M. J., Brady, R. O., and Quarles, R. H.: Metabolism of a myelin-associated glycoprotein in developing rat brain, Brain Res. in press.

10. Matthieu, J.-M., Brady, R. O. and Quarles, R. H.: Anomalies of myelin-associated glycoproteins in Quaking mice, J. Neurochem. in press.

11. Matthieu, J.-M., Brady, R. O. and Quarles, R. H.: Developmental change in a myelin-associated glycoprotein: A comparative study in rodents, Dev. Biol., in press.

12. Matthieu, J.-M., Quarles, R. H., Webster, H. deF., Hogan, E. and Brady, R. O.: Characterization of the fraction obtained from the CNS of Jimpy mice by a procedure for myelin isolation, J. Neurochem., in press.

Serial No. NDS(I)-73 DMNB/CIT 2024(c)
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of Plasma Partitioned Lipids, Lipoprotein and Blood Viscosity in Patients with Transient or Definitive Strokes and in Family Members with Hyperlipidemia

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Jan K. Steusing

Cooperating Units: In negotiating phase

Man Years:

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

Objectives: Cerebrovascular disease is one of the most common causes of neurological morbidity and mortality among senior citizens; the condition also occurs with a substantial frequency during middle adult years and low frequency under 20 years of age. Atherosclerotic plaques are the most common pathological lesions encountered in the occluded arteries. Of various factors predisposing to atherosclerosis is persistent elevation of plasma lipids (whether exogenous - dietary, or endogenous - hereditary hyperlipidemia) and abnormal patterns of lipoproteins. It is our intention to study the content and composition of partitioned lipids and lipoproteins in patients with transient or definitive strokes and in persons at high risk such as members of families with hyperlipidemia. Special dietary measures will be made available to these patients and arrangements will be made for their follow-up.

Material: 37 patients with transient cerebral ischemic attacks or definitive strokes were examined in ambulatory setting and fasting blood drawn.

Methods Employed:
1. Detailed personal and family medical history including dietary habits.
2. General medical and neurological examinations.

3. Special examination of the retinal blood vessels and peripheral arteries.
4. Radiological, electroencephalographic, electrocardiographic, blood viscosity studies and usual routine laboratory surveys of blood, urine and CSF.
5. Special tests will include - when indicated for diagnostic or planned therapeutic reasons - cerebral angiography and brain scan.
6. Determination of plasma partitioned lipids using established chemical procedures as employed by us in studies ofhigh fat diet in children administered for treatment of epilepsy. Determination of plasma lipoproteins by unidimentional paper electrophoresis supplemented by polyacrylamide gel electrophoresis and density gradient centrifugation; the last procedure will be used only in special instances.
7. Long range management and need for follow-up will be discussed with the patients and appropriate arrangements made.

Major Findings: This project is currently in a developmental stage and several new parameters of study will be considered. Blood viscosity and especially devised performance tests will be among the first to be added. Each patient's profiles of serum partitioned lipids including separation of phospholipids as well as profiles of lipoprotein are in progress.

During the past several months, 37 patients with stroke or transient ischemic attacks were examined in ambulatory setting and 14-hour fasting blood drawn. The separated plasma was divided into two samples; one of these was used for immediate determination for lipoproteins, the second sample was kept frozen until appropriate time when partitioned lipids and separations of phospholipids (TLC) could be performed. Out of 37 patients thus studied, twelve, or 32%, had hyperlipidemia in a form of abnormally high plasma cholesterol or triglycerides or both (the elevation had to be more than 3 S.D. over the mean value for the age to be considered abnormally high). Of these twelve patients, nine or 24%, had also abnormal lipoprotein patterns, mainly of Type II, III and rarely of Type IV hyperlipoproteinemia. It needs to be stated that a sizable proportion of the 37 patients with stroke so studied derived from the families with hyperlipidemia. It is possible that some of the patients with excessive plasma lipid values may have an exogenous type of hyperlipidemia; this necessitates further studies, including placement on special diet for 2-4 weeks.

It is of interest that a few patients with Fabry's disease and with Neimann-Pick disease who were studied for different reasons, had also abnormal serum lipids. This may prove of value in following the live history of these particular diseases.

Significance to Bio-Medical Research and the Program of the Institute: There is an obvious need for additional research on cerebrovascular disease. Since the relationship between the severity of atherosclerosis and cerebral symptoms is reasonably well established, it will be fruitful to evaluate the handling of high fat diet ingested by the subjects with marked differences in fat metabolism. This could lead to the dietary modifications during early life, and may provide one mechanism for reducing the incidence of cerebrovascular damage.

Proposed Course of the Project: Although begun, this project is still in a process of development and further expansion.

Honors and Awards: None

Publications: None - this project is still in a phase of development.

Serial No. NDS(1)-73 DMNB/CIT 2103
1. Developmental and Metabolic Neurology Branch
2. Section on Clinical Investigations and Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Intravenous Infusion of Ceramidetrihexosidase in Patients with Fabry's Disease and Glucocerebrosidase in Patients with Gaucher's Disease; a Therapeutic Intent.

Previous Serial Number: NONE

Principal Investigators: Anatole S. Dekaban, M.D. and Roscoe O. Brady, M.D.

Other Investigators: J. F. Tallman, Ph.D., P. G. Pentchev, Ph.D., A. E. Gal, Ph.D., A. W. Zimmerman, M.D., M. Zelkowitz, M.D.; J. M. Quirk.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.9
Other:	0.1

Project Description:

Objectives: Fabry's and Gaucher's diseases are serious, life-threatening hereditary disorders of lipid metabolism. Previously no satisfactory therapy was available and these patients are greatly handicapped; and in addition, those with Fabry's suffer from intractable pain. Patients with Gaucher's disease accumulate cerebroside excessively in their organs. As a result of enzyme deficiency glucocerebrosidase and the patients with Fabry's disease store excessive amounts of ceramidetrihexoside as a result of deficiency of ceramidetrihexosidase. There is a direct or indirect involvement of the nervous system in both these diseases. We have isolated and purified ceramidetrihexosidase and glucocerebrosidase with the view of eventually using these enzymes as replacement therapy for Fabry's disease and Gaucher's disease respectively.

Methods: Active enzymes, α-galactosidase and ceramidetrihexosidase glucocerebrosidase are obtained from human placenta by extraction and fractionation with ammonium sulfate. They are subsequently purified by passages on Sephadex G-200 columns, ion exchange chromatography including DEAE and Sephadex cation exchange columns. The final enzyme proteins are homogeneous and pure. The enzymes are suspended in albuminated saline and submitted to the NIH Pharmaceutical Development Service for study of

sterility and pyrogenicity in order to obtain their clearance for intravenous administration to the patients.

Single injections of 4-6 mg thus prepared enzyme in 10 to 20 ml saline will be given intravenously to the respective patients over the period of one minute. Subsequently, samples of blood will be taken for determination of enzyme activity and substrate content. The total blood volume taken during 24-hours will not exceed 80 ml in adult and adolescent patients.

In addition, two liver needle biopsies will be performed, one prior to injection of the enzyme and the second 12-24 hours after the injection. This is absolutely necessary in order to determine enzyme activity in this organ (does this enzyme reach the hepatic cells and how long does it remain active?) as well as to estimate the change in intracellar accumulation of the storage material as a result of infused enzyme's activity.

Major Findings: The infusion of ceramidetrihexosidase into two patients with Fabry's disease caused a dramative decrease in the elevated level of circulating ceramidetrihexoside in each patient. There was a gradual return in the quantity of this lipid in the plasma to preinfusion levels by 48 hours after injection of the enzyme. The injection of purified glucocerebrosidase into two patients with Gaucher's disease caused a 26% reduction in the amount of accumulated glucocerebroside in the liver of the recipients. Furthermore the elevated quantity of glucocerebroside in the erythrocytes of these patients was reduced to normal by 48 hours after the enzyme was infused. The amount of glucocerebroside in the red blood cells persisted at a reduced level for four months following administration of glucocerebrosidase.

Proposed Course: We shall undertake investigations of the long-term effects of enzyme replacement in patients with Fabry's disease and Gaucher's disease. Particular attention will be devoted to assessment of the clinical results of this therapy and the possibility of antibody formation by the recipients will be critically assessed.

Publications:

1. Brady, R. O., Tallman, J. F., Johnson, W. G., Gal, A. E., Leahy, W. R., Quirk, J. M., and Dekaban, A. S. Replacement therapy for inherited enzyme deficiency: Use of purified ceramidetrihexosidase in Fabry's disease. New Engl. J. Med. 289: 9-14, 1973.

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Neuropathology and Neuroanatomical Sciences, IR
National Institute of Neurological Diseases and Stroke

Igor Klatzo, Chief

The main research effort of this laboratory is directed towards understanding the basic pathological mechanisms operative in debilitating neurological conditions such as stroke and demyelinating diseases. The approach is to investigate structural, biochemical, and physiological phenomena in the nervous system under normal conditions and in experimental models imitating the mentioned disorders in humans.

With regard to research problems related to stroke, the Section on Cerebrovascular Pathology has been serving as the coordinating center for a collaborative effort involving other sections of the laboratory. Since stroke is related to disturbance of cerebral circulation and particularly to cerebral ischemia, the latter condition has been studied in Mongolian gerbils (Meriones unguiculatus) in which, due to frequent incompleteness of the circle of Willis, cerebral ischemia can be produced (in ± 30% of animals) by ligation of a single common carotid artery.

In the area of cerebral ischemia, a concept of "no-reflow" (NR) phenomenon was introduced to describe a secondary impairment of cerebrovascular circulation in areas of the brain submitted to ischemia. Our studies have been undertaken because of an implication of the potential clinical significance of NR phenomenon with regard to the possibility that such secondary impairment of cerebral circulation may ultimately determine the severity and the extent of ischemic brain damage after release of the original arterial obstruction, a matter of potential clinical significance. Contrary to suggestions by some investigators that swelling of endothelium and of perivascular astrocytes might be responsible for the NR phenomenon, our studies have indicated a close relationship of NR to the fluctuations in the systemic blood pressure. The release of a carotid clipping was associated with a rapid, marked drop in systemic blood pressure and a 100% incidence of NR phenomenon (30 seconds after release), whereas, 10 minutes or longer after release when blood pressure would return to almost prerelease levels NR phenomenon was no longer demonstrable. Further evidence of dependence between the incidence of NR phenomenon and the fall in systemic blood pressure was provided by data on the effect of certain sympathomimetic substances. Epinephrine which was most effective in preventing the drop in blood pressure following release of carotid occlusion was also most effective in prevention of the NR phenomenon. This opens a possibility of using pharmacological agents for control in clinical situations.

One of the complications of cerebral ischemia is the development of brain edema. Two main types of brain edema are the cytotoxic and vasogenic varieties, the behavior of the blood-brain barrier (BBB) being one of the main criterions by which these types can be differentiated. Our studies on elucidation of the nature of ischemic brain edema suggested that primarily an abnormal accumulation of fluid takes place intracellularly, indicating edema of the cytotoxic type. Only in later stages when there is damage to cerebral blood vessels and their permeability to BBB tracers is increased does leakage of serum components contribute a vasogenic component to the overall picture of brain edema. These findings might be of importance in designing proper treatment for patients suffering from cerebral ischemia.

The incidence of the BBB damage, evidenced by Evans blue staining of the brain tissue, was related to the duration of ischemia and to the duration of release following carotid occlusion. Thus, e.g., in gerbils subjected to 6 hours ischemia all animals showed the BBB damage one hour after release of occlusion. On the other hand, gerbils with one hour carotid occlusion showed 100% incidence of the BBB damage only 20 hours following release of carotid clipping. Such delayed type of ischemic damage was demonstrable after limited duration of ischemia also with regard to histological changes. These experimental observations provide a new insight for interpretation of clinical course in patients with cerebral ischemia. Of further clinical relevance is the observation that raising the systemic blood pressure accelerates significantly the BBB damage, the appearance of histological changes, and the development of ischemic brain edema.

In view of several reports implicating the biogenic amines in the dynamics of brain edema, a study was undertaken to elucidate these assumptions. Cats subjected to cortical cold injury for production of experimental vasogenic brain edema showed the blood vessels, confined exclusively to the area of injury of the cerebral cortex, exhibiting a bright, yellowish-green fluorescence in the sections prepared by the Falck method. Microspectrofluorometry carried out on these blood vessels revealed that this fluorescence had the excitation-emission spectra and photodecomposition rate characteristic for serotonin. Assaying quantitatively the area of cortical injury and the edematous white matter using a modification of the method of Maickel demonstrated about four-fold increase of serotonin in the injured cortex. Using ^{3}H labeling of platelet serotonin, it was possible to ascertain the platelet origin of serotonin in the injured cortical blood vessels. With regard to the clinical significance of these observations, it is possible that this phenomenon may occur in vascular injuries other than those produced by cold and it may be expected in the injuries associated with thrombus formation. The possibility that serotonin acting on cerebral blood vessels may affect their permeability is supported by recent observations from our laboratory that serotonin and other biogenic amines reaching arterioles and venules from the brain parenchyma may alter their permeability to protein tracers. It is conceivable that the presence of appreciable amounts of serotonin in injured blood vessels may significantly affect the duration and intensity of vascular leakage, which is a crucial factor in the dynamics of vasogenic brain edema.

In the Section on Neurocytobiology, in another investigation on cerebral ischemia, an increase in facilitated transport of ^{14}C 2-deoxy-D-glucose, as well as of ^{14}C 3-O-methyl-D-glucose, was demonstrated in both sensitive (with cerebral ischemic damage) and nonsensitive (without cerebral damage) gerbils after various periods of carotid occlusion. The facilitated transport was evident from assays on self-inhibition, as well as, on inhibition by phlorizin. An increase in uptake of ^{14}C sucrose was demonstrable only in gerbils with severe ischemic injury of blood vessels (e.g., 18 hours occlusion), evident by leakage of the BBB tracers.

Different results were obtained in studying the effects of hypoxia and carbon dioxide tension on glucose transport. Abrupt lowering of oxygen tension below 18% in rabbits resulted in a 40-45% decrease of both 2-deoxy-D-glucose and 3-O-methyl-D-glucose uptake. Similar results were obtained in increase in arterial pCO_2 with respiratory acidosis. On the other hand, hypocapnia with respiratory alkalosis caused an increased uptake of 3-O-methyl-D-glucose. Since glucose is the basic cerebral nutrient, elucidation of factors which affect its uptake by the brain should help in understanding the pathophysiologic processes involved in carebral ischemia and hypoxia, and may suggest some means for limiting the brain tissue damage resulting from these conditions.

In order to get some insight into which cellular elements may be involved, a study on facilitated glucose transport was undertaken in conditions of tissue culture. This is the first research project started in our new tissue culture laboratory, in spite of the considerable difficulty of not having the assistance of a qualified technician. Newborn rat cerebellar explants were grown on collagen in Maximow's assemblies. The cultures were incubated with ^{3}H O-methyl-D-glucose. The cerebellar tissue cultures uptake of glucose was considerably lower in the cultures incubated with the mixture of labeled and unlabeled tracer than with medium containing only labeled hexose. This self-inhibition of glucose uptake suggests that uptake of nonmetabolizable O-methyl-D-glucose occurred by a facilitated transport mechanism. The assessment as to which cells are mainly involved in this uptake is being presently carried out using radioautography.

Since the brain endothelium may play a paramount role in the blood-brain transport of essential nutrients such as glucose, the second project involving tissue culture concerns a study of cerebral endothelium grown in vitro. In preliminary observations, it was possible to observe an outgrowth of endothelial cells from the cerebellar and meningeal explants. The endothelial cells appeared to grow in parallel chains of cells, simulating capillaries forming occasionally small loops and were distinguishable by form and pattern from other cells. The endothelial cells in cultures will be subjected to histochemical and biochemical observations, as well as, a study with the electron microscope.

To provide a basis for understanding of biochemical events in cerebral ischemia and hypoxia, the Section on Cellular Neurochemistry is concerned with carbohydrate metabolism and its regulation, and the role of cyclic

nucleotides in the central nervous system. These areas are being explored in mammalian systems and in tissue culture. The specific areas of interest now being investigated are as follows:

The activity and properties of glycogen synthetase in the brain and in glioma and neuroblastoma cultures are being examined. The interconversion of the active form of synthetase (I) and the inactive form (D) is regulated by protein kinase which in turn is activated by cyclic AMP. Further study concerns the effects of drugs which are known to modify levels of cyclic AMP. For example, norepinephrine releases cyclic AMP which activates protein kinase. A series of events follows in which (a) the protein kinase activates phosphorylase b kinase (b) the phosphorylase b kinase converts phosphorylase b to a which is the active form. Concomitantly, the protein kinase (c) phosphorylates glycogen synthetase I converting it to the inactive form D. The events thus regulated by cyclic AMP assure that glycogen can be readily mobilized, and glycogen synthesis will be curtailed when energy stores are required. Electroconvulsive shock in mice has also been investigated with regard to these two enzymes regulating glycogen metabolism, the cyclic nucleotide levels and intermediary metabolites.

Both cyclic AMP and cyclic GMP which appear to have opposite effects in the central nervous system are being measured in normal mice, electrically stimulated mice and animals given drugs which are known to affect the enzymes responsible for cyclic nucleotide synthesis and degradation.

By using glioma and neuroblastoma lines in pure culture, the cell lines can be studied separately. It was observed that both lines excrete pyruvate and lactate into the medium as long as glucose is abundant in the medium. If the glucose decreases below an optimum level, the pyruvate and lactate are taken up by the cells and metabolized. Further studies on these tissue cultures are planned to determine the differences in metabolism, responses to hormones, and responses to drugs which act on the central nervous system.

Besides endothelium, another cellular element which has been suspected in transport mechanisms in the brain is the astroglia. In the Section on Functional Neuroanatomy, application of the freeze-fracture technique was concentrated on study of the membranes of astrocytes and a striking intramembranous specialization was found where these membranes faced blood vessels, cerebrospinal fluid or large neuronal cell bodies. This finding suggest that astrocytes might transport or store certain substances exchanged between neurons and blood. Otherwise, the Section on the Functional Neuroanatomy has completed the study on four important aspects of synaptic structure: (1) the site and nature of the interaction of synaptic vesicles with the presynaptic membrane, (2) the location of structures in postsynaptic membrane that are probably related to receptors or ion channels, (3) the formation of synapses in a genetically deficient mouse, and (4) the factors preventing new synapse formation in certain regions of the brain.

The sites of discharge of synaptic vesicles have been identified in several types of synapses and it has been shown that discharge requires fusion of the vesicle membrane with the plasmalemma. The vesicles that

contain the readily releaseable pool of transmitter have been identified and it is apparent that these vesicles always have a special relationship to a peculiar fuzz on the inside of the plasma membrane and to a special array of particulate materials within the plasmalemma. Special arrays of particles are also associated with the postsynaptic membrane. Furthermore, each of the several chemical types of synapse that have been studied appears to be characterized by specific structures in the postsynaptic membrane. At a more complex level of the organization of synaptic connections in the mammalian central nervous system, it has been found that in certain genetic defects specific synaptic connections are missing, although both the pre- and postsynaptic processes are present and may make other synaptic connections. New synapse formation after brain injury in the adult mammal has been studied and it is apparent that certain interrupted synapses are not replaced by axonal sprouting from adjacent synapses. These investigations contributing to understanding the detailed structure of synaptic vesicle release offer the possibility to determine the state of synaptic activity and whether this state is normal or pathological. This knowledge should be of great use in evaluating synapses from epileptogenic foci. The other studies are relevant to determining the factors leading to, or preventing, formation of new synapses in normal and diseased or injured central nervous systems.

In the Section on Neurocytology, the investigations are concerned mainly with changes inside cell membranes of vertebrate and invertebrate nervous system during degeneration and regeneration of peripheral and cranial nerves, in crayfish and rats, cationic stimulation of nerve sheath smooth muscle and neurosecretory cells in the marine gastropod, Aplysia and in higher forms, and alterations inside tight junctions during their osmotic opening in rodents. All of these intramembranous changes are being followed by electronmicroscopy of frozen-cleaved specimens.

A common event, discernible because of the panoramic view provided by freeze-fractured membranes, is the increase in contact sites between vesicles or caveolae and the cell membrane. Thus, in the glial cells accompanying regeneration in crayfish, linear arrays of small, circular depressions or elevations become prominent. In KCl stimulated smooth muscle cells in the ganglia of Aplysia, there is a like increase in similar but larger sites inside the muscle cell membranes. These sites are stable, being unchanged after fixation at low pH or in cells frozen without prior fixation or cryoprotection. In the glial cells, the contact sites are probably involved in a heightened exchange of fluid and solute across the cell membrane. The glial sites might thus participate in the exchange of metabolites between neuronal and glial processes. In smooth muscle, the contact sites belong to membrane pits that are probably not pinocytotic but rather act like a transverse tubule system involved in excitation-contraction coupling.

In the Section on Experimental Neuropathology further investigations were carried out on the specificity of histological changes in neurons observed in ischemia and anoxia. In the experimental model of oil embolisation, it became evident that present concepts about the characteristics of neuronal changes in anoxia, the shrunken ischemic neuron, must be revised.

In one series of experiments, the morphological and tinctorial similarities between the neuronal changes regarded as the prototype of anoxic changes, and the artifactitious changes of normal neurons were pointed out. Accordingly, the so-called ischemic neuron in infarcted tissue is regarded as the homologue of the dark neuron in normal tissue, both affected by post-mortem handling of the unfixed brain. In another set of experiments with compression of the cerebral cortex, many neurons displayed a pale cytoplasm as the consequence of rapid disintegration of proteins, while the shrunken ischemic cell type was absent.

In the research area on demyelinating diseases benefiting from collaborative and administrative support, the laboratory's multiple sclerosis research program has expanded. Substantial progress has already been achieved on an objective of major importance, the development of a simple model system of CNS myelin for studying the morphological parameters of demyelination and remyelination. In the optic nerves of Xenopus tadpoles, the relationships of axons, myelin sheaths, and glial cells, their fine structure, and the morphological features of myelin formation all resemble those of mammalian experimental animals and humans. Xenopus tadpoles are transparent; their optic nerves can be observed directly and it is relatively simple to transect them or expose them to injected materials without injuring adjacent vessels, nerves, or muscles. Whole mounts of optic nerves can be prepared quickly and they can be optically sectioned by using differential-interference contrast illumination. Using this quick, simple procedure it is possible to survey many myelin sheaths within this CNS tract over long distances. Demyelinating lesions, produced by sensitized lymphocytes or serum from animals sick with EAE, have been identified and counted without the time-consuming effort of preparing and surveying large numbers of stained sections mounted on glass slides. Currently, this model system is being used to test the demyelinating activity of sera and CSF from patients with multiple sclerosis. Also, CNS myelin constituents, such as basic protein, cerebrosides, etc., will be used to prepare immune sera for similar tests.

Model systems suitable for studying factors affecting the capacity of CNS axons and their myelin sheaths to regenerate after injury are also of major importance in paraplegia research programs. Tadpole optic nerve is almost the only heavily myelinated CNS tract that can be easily transected under direct observation with insignificant injury to its blood supply and adjacent structures. Distal to the transection site, degeneration of axons and myelin sheaths is apparent within 16 hours and is complete within 2 weeks. Remyelination of some distally located, regenerating axons begins 3 weeks after transection and progresses rapidly thereafter. The rapid pace of degeneration and regeneration in tadpole optic nerves suggests that they are also an excellent model system for testing the effects of hormones, drugs, and other substances on regeneration of myelinated axons in CNS tracts.

Serial No. NDS(I)-71 LNNS/CNC 1884
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Brain Glycogen Synthetase

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: Joan P. Schwartz, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: To determine the role of glycogen synthetase in glycogen formation in brain in normal and experimental states.

Methods Employed: Two methods are employed for the measurement of enzyme activity. The first is a coupled enzyme assay using pyruvate kinase and lactate dehydrogenase to measure uridine diphosphate which is formed in stoichiometric amounts with the glucose incorporated into glycogen from uridine diphosphoglucose. The second assay utilizes a radioisotope precursor, ^{14}C-uridine diphosphoglucose, and the incorporation of the radioactive glucose moiety into glycogen is measured.

Major Findings: The requirement for preincubation with glycogen for full activity of the purified brain enzymes was investigated. The enzyme-substrate complex was studied using sucrose-density gradients in the ultracentrifuge. The molar ratio of glycogen : enzyme indicated that the enzyme preferentially binds to low molecular weight polysaccharides.

Significance: The glycogen synthetase activity is dependent on the form in which it exists as well as the concentration of substrates and modifiers. The regulation of the activity in brain is of great importance to understand the accumulation of glycogen in anesthesia and trauma, and its depletion in early trauma and ischemia.

Proposed Course of Project: It is anticipated that the work will be written up and the project concluded in the coming year.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/CNC 1885
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The kinetics of brain glycogen

Previous Serial Number: SAME

Principal Investigator: Janet V. Passonneau, Ph.D.

Other Investigators: David Lust, Ph.D.
Vivian Lauderdale, M.S.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.1
Other:	0.3

Project Description:

Objectives: To further explore factors in the turnover, accumulation, and disappearance of glycogen in the brain. To determine the effects of trauma, drugs, and altered physiological states such as hypothermia or drug administration on glycogen metabolism.

Methods Employed: A stab wound injury was made in one cerebral hemisphere of mouse brain and the contralateral side served as control. Glycogen content was measured at intervals after injury, as well as related metabolites and enzymes. These included glucose, glucose-6-P, uridine diphosphoglucose, cyclic AMP, glycogen phosphorylase and glycogen synthetase.

Major Findings: Following the study of glycogen turnover in brain, further experiments were carried out to determine how glycogen metabolism was altered by trauma (stab wound).

The turnover of brain glycogen increased greatly in the early period after injury (up to 2 hrs after injury). At later periods after injury (24 hrs) the incorporation of radioactivity from ^{14}C-glucose precursor was not different from controls. The rate of disappearance of label from cerebral glycogen indicated decreased glycogenolysis 24 hrs after injury. The percent of phosphorylase in the active form (a) decreased at 24 hrs after injury indicating decreased breakdown of glycogen.

The metabolic rate of brain was decreased at one minute after injury. Subsequently at 10 and 60 minutes after injury the metabolic rate was 1.5 times greater than that of controls. After 24 hrs, the metabolic rate in the injured brain was like that of normal brain.

Various drugs were used in order to determine the mechanism for the trauma-induced increase in cyclic AMP concentration in the brain. Following the stab wound injury, cyclic AMP levels increased 7-fold in the traumatized region. Cyclic AMP is known to increase in brain when catecholamine levels have been increased due to experimental treatment. In the present study we have found that antiadrenergic agents do not alter the increase in cyclic AMP seen in the cortex after trauma. These agents included dichlorisoproterenol, pronethalol and reserpine. An antihistamic drug, diphenhydramine, decreased the concentration of cyclic AMP following injury. Theophylline, phenothiazines, and chlorpromazine all depressed the cyclic AMP increase due to injury. Hypothermia delayed or decreased the post-trauma elevation of cyclic AMP.

The findings are consistent with an adenosine-mediated increase in cyclic AMP in the brain, as a consequence of stab wound with the concomitant ischemia and membrane depolarization. Theophylline inhibits adenosine effects, and chlorpromazine diminishes the effects of depolarizing agents.

In the process of studying glycogen metabolism, a new method for the measurement of total glycogen was devised. The method has been accepted for publication in Analytical Biochemistry.

Significance: Brain glycogen levels have been shown to increase in various traumatic and experimental states. The changes in turnover rate in anesthesia and trauma are of great concern in order to understand the changes in metabolism that occur in pathological states.

The effect of trauma (stab wound) on brain glycogen was studied. There was an initial drop in glycogen content (up to 10 min) followed by an increase which was maintained for 24 hours. The active form of glycogen synthetase decreased at 10 min and increased at later intervals. Glycogen phosphorylase was converted to the active form following trauma which produced an increase in cyclic AMP. The metabolic rate decreased at 1 min following trauma, and then increased over normal values. At 24 hours, the metabolic rate was back to normal. The turnover of cerebral glycogen as followed with ^{14}C-glucose was accelerated 1 hr after injury, and decreased 24 hours after injury. These findings indicate at least 2 distinct stages of glycogen metabolism following injury. First, there is a decrease in glycogen content, an accelerated turnover time and an increase in phosphorylase *a*. Later, the turnover time is increased, glycogen accumulates and there is a decrease in phorphorylase *a*.

Proposed Course of Project: Further studies are being made on the effect of electroconvulsive shock, insulin, and experimental brain edema on the rate of incorporation of ^{14}C-glucose into brain glycogen and the subsequent loss of label.

Honors and Awards: None

Publications:

Watanabe, H. and Passonneau, J.V.: The effect of trauma on cerebral glycogen and related metabolites and enzymes. Brain Res. 66: 147-159, 1974.

Passonneau, J.V. and Lauderdale, V.R.: A comparison of three methods of glycogen measurement in tissues. Anal. Biochem. (in press).

Serial No. NDS(I)-72 LNNS/CNC 1942
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The role of adenosine 3',5' cyclic monophosphate (cyclic AMP) and guanosine 3',5' cyclic monophosphate (cyclic GMP) in the central nervous system.

Previous Serial Number: SAME

Principal Investigator: W. David Lust, Ph.D.

Other Investigators: Janet V. Passonneau, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To determine if either pharmacological or physiological alterations of brain metabolism would affect the steady state levels of cyclic AMP and/or cyclic GMP in vivo.

Methods Employed: Mice were rapidly frozen in liquid nitrogen at the appropriate times following treatment. The brains were removed at -25°, weighed and extracted in perchloric acid. The neutralized PCA extracts were used in all subsequent metabolic measurements. Cyclic GMP was measured by an enzymic-cycling technique and cyclic AMP was measured by a radioactive binding assay.

Electroconvulsive shock (ECS) was applied by corneal electrodes at an intensity of 50 mA for a duration of 0.2 sec. The electroshock produces a convulsive response manifested by a) a tonic extensor phase (0-15 sec), b) an intermittent clonic phase (15-30 sec) and c) a postictal depressive phase ($>$ 30 sec).

In the thermal experiments, the mice were rendered a) hypothermic (20°) by forcing them to swim for 1-2 min in a water bath followed by a 10 min stay in a cold chamber, and b) hyperthermic (41°) by placing them under a heat lamp. Rectal temperatures were monitored with a tele-thermometer.

Major Findings: The continuation of the work on electroconvulsive shock (ECS) and ischemia (produced by decapitation) confirmed our previous findings that the levels of cyclic AMP increase rapidly following both treatments in all areas of the brain tested. Although the concentration of cyclic GMP does not significantly change during the 60 seconds following decapitation, this cyclophosphate is elevated after ECS in both the cerebellum and the forebrain. The major difference between the cyclic nucleotide responses is that the maximal change in cyclic AMP occurs at 30 seconds and precedes the cyclic GMP peak by approximately 30 seconds. Relating these responses to convulsive behavior, it is apparent that the major cyclic AMP changes occur during the excitable states (tonic, 0-15 seconds and clonic, 15-30 seconds), and cyclic GMP during the depressive stage (postictal, greater than 30 seconds). Although the levels of cyclic nucleotides drop rapidly following their maximal ECS-induced changes, the restoration of cyclic AMP and cyclic GMP to control levels was not reached by 240 seconds. In addition, the cyclic AMP response to ECS differs in the cerebral cortex and the cerebellum. The cyclic AMP response in the cerebral cortex is 2-3 fold greater than that in the cerebellum and the peak response occurs at 45 sec post-ECS or some 30 seconds after the cerebellar maximum. In contrast, the cyclic AMP increase following decapitation is greater in the cerebellum than in the cerebral cortex.

To determine what effect altered body temperature would have on cyclic nucleotides, mice were rendered hypothermic (20°) or hyperthermic (41°) as described in the methods. In hypothermic mice, the levels of cyclic AMP were lower than normothermic values in both the cerebellum and the cerebral cortex. The levels of cyclic GMP were not affected. Further, the cyclic AMP increases following either decapitation or ECS were significantly reduced. In hyperthermic mice, both cyclic nucleotides were unchanged in NIH mice; however, the concentrations of cyclic GMP increased 2-fold in the DBA-2N strain of mice. Hypothermia delays the cyclic AMP response to ECS in the cerebral cortex. Although the peak levels resulting from ECS are approximately the same in both hypothermic and normothermic mice, the hypothermic maximum occurs at 240 sec post-ECS, some 3 min after the normothermic peak. From the measurements of ATP, phosphocreatine, glucose and phosphorylase a, post-ECS, it appears as is the case with cyclic AMP that hypothermia only affects the time course and not the magnitude of these metabolic changes.

In pharmacological studies, phenobarbital, papaverine and $MgSO_4$ significantly decreased the levels of cerebellar cyclic GMP. Phenobarbital (100 mg/kg IP) lowered cyclic GMP within five minutes and the levels remained at 20% of control for up to 90 minutes. Of all the drugs tested, only chlorpromazine and trifluopromazine lowered cyclic AMP levels. Pretreatment with two drugs, amphetamine (10 mg/kg IP) which elevates GMP 2-fold, or phenobarbital (20 mg/kg IP) which decreases cyclic GMP 50%, had no effect on the ECS-induced increase in cyclic nucleotides, in spite of significant alterations in overt convulsive behavior.

Using 4 different fixation techniques, the levels of cyclic AMP were lowest in the microwave irradiated brain with increasing amounts in brains which were freeze-blown, frozen by immersion and frozen following decapitation. In comparison, the degree of anoxia as determined by the changes in ATP, phosphocreatine and lactate was lowest in the freeze-blown brain, with increasing anoxia in brains which were frozen by immersion, irradiated, and frozen following decapitation.

Significance: Both the ECS and pharmacological data favor the argument that cyclic AMP and cyclic GMP are independently regulated in vivo. The possibility of a direct relationship between elevated cyclic GMP and depression seems remote, since pharmacologically-induced depression either reduced or had no effect on cyclic GMP levels. There is an increasing amount of evidence favoring an association between cyclic AMP and the aminergic system and between cyclic GMP and the cholinergic system. Thus, neural events following such treatments as ECS or brain injury might be defined by measuring the cyclic nucleotides. For example, the aminergic system would predominate in the first thirty seconds after ECS and the cholinergic system from thirty seconds on.

The ECS- and anoxia-induced changes in brain cyclic AMP exhibit regional variation. Thus, while the cerebellum is more sensitive to the stimulus of anoxia, the cerebral cortex is more responsive to the electrical stimulus. Although hypothermia does slow metabolic events in the brain, it is apparent that only the temporal relationships and not the magnitude of response are affected.

Since cyclic AMP increases rapidly in the brain following decapitation, low cyclic AMP levels have been equated with rapid fixation. However, following microwave irradiation low levels of cyclic AMP were obtained although the irradiated brains were severely anoxic by other criteria. Of the fixation methods tested, freeze-blowing and freezing intact most closely approximate the in vivo state.

Proposed Course of Project: More extensive experiments on the ECS-dependent rise of cyclic nucleotides are being undertaken in other areas of the brain. Using the appropriate pharmacological antagonists, the cyclic nucleotide-putative neurotransmitter relationship after ECS may be elaborated. Further, the structure activity relationship of the phenobarbital-induced decrease in cyclic GMP is being investigated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/CNC 2006
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of metabolism in glioma and neuroblastoma cell lines

Previous Serial Number: SAME

Principal Investigator: Joan P. Schwartz, Ph.D.

Other Investigators: W. D. Lust, Ph.D.
J. V. Passonneau, Ph.D.
V. R. Lauderdale, M.S.

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	1.7
Other:	0.2

Project Description:

Objectives: To investigate the regulation of metabolism in a glioma and a neuroblastoma cell line.

Methods Employed: The cells are being grown in a CO_2 incubator in the laboratory. Homogenates of the cells are analyzed for various metabolites, such as cyclic AMP, cyclic GMP, glycogen and pyridine nucleotides, as well as for enzymes such as the adenylate cyclase, cAMP phosphodiesterase and glycogen synthetase and phosphorylase. All of these analytical methods have been used in the laboratory on whole brain and are thus easily adaptable for use with the cells. In addition, the effect of various agents on the morphology of the cells will be undertaken when a phase microscope becomes available.

Major Findings: The glioma cells contain an adenylate cyclase which is responsive to catecholamines such as norepinephrine. Activation of the cyclase by norepinephrine elevates the intracellular cyclic AMP level, resulting in the breakdown of glycogen due to inactivation of the glycogen synthetase and activation of the phosphorylase (Browning, E.T., Schwartz, J.P., and Breckenridge, B.M.: Mol. Pharmacol. 10: 162, (1974)). The expression of the norepinephrine receptor has been shown to require cell contact. Activity

of the cyclic AMP phosphodiesterase can also be regulated, by growth of the cells in either dibutyryl cAMP or in bromo-deoxy-uridine, an agent which causes dedifferentiation (Schwartz, J.P., Morris, N.R. and Breckenridge, B.M.: J. Biol. Chem. 248: 2699, (1973)). Treatment of the glioma cells with norepinephrine not only results in short-term effects, but also causes an induction of the phosphodiesterase. The induction has been shown to be mediated by cyclic AMP via the β-receptor and to require new protein synthesis.

In addition to cyclic AMP metabolism, a study of the energy metabolism of both cell lines has been carried out. The kinetics of glucose uptake, of glycogen breakdown, and of pyruvate and lactate efflux and uptake have been studied. The effect of thiamine deficiency, brought about by growth without thiamine or in the presence of the anti-thiamine drug pyrithiamine, on the above parameters has also been examined.

Significance: A study of the regulation of metabolism in the brain is complicated by the presence of several cell types and the inability to determine in which cells metabolic alterations are occurring. Such studies are facilitated by the use of the two distinct cell lines, the glioma (rat C6 line) and the neuroblastoma (mouse C1300 line), because regulation can be studied in each cell line separately. Until these cell lines became available, for example, it had not been realized that glial cells might be hormone-responsive. Such results suggest that the glial cells may have a greater involvement in brain function than the supportive one originally envisaged. Although these two cell lines are tumor cells, they offer a first approach to the problem of regulation of metabolism in the brain, which might be followed up in normal brain cells when the isolation techniques for glial and neuronal cells have been perfected.

Proposed Course of Project: A thorough investigation of the regulation of cyclic AMP and cyclic GMP levels, of glycogen metabolism and other energy-related parameters, and of pyridine nucleotide ratios will be undertaken, in both cell lines. Studies on ion transport are also planned, as are morphological studies of the effects of various agents and growth conditions.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/CNC 2007
1. Neuropathology & Neuroanatomical Sciences
2. Section on Cellular Neurochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of the NAD^+/NADH ratio in normal and virally-transformed tumor cell lines

Previous Serial Number: SAME

Principal Investigator: Joan P. Schwartz, Ph.D.

Other Investigators: Janet V. Passonneau, Ph.D.
George S. Johnson, Ph.D.
Ira Pastan, M.D.

Cooperating Units: Laboratory of Molecular Biology, NCI

Man Years:

Total	0.1
Professional:	0.1
Other:	0

Project Description:

Objectives: To investigate factors which regulate the ratio of total and free NAD^+/NADH in normal and transformed cell lines.

Methods Employed: The cells are homogenized following growth under various conditions. The homogenates are analyzed for NAD^+ and NADH by a fluorometric procedure which involves enzymatic cycling to provide the necessary sensitivity.

Major Findings: The total NAD^+/NADH ratio is low (2-3) in log phase normal cells but rises with confluency to 10-12. However, no such rise in the ratio is seen in the transformed tumor cells as they become confluent. Corresponding changes were found in the ratio of the free pyridine nucleotides. These changes could not be correlated with intracellular cyclic AMP levels. Nor did growth-stimulating compounds such as serum, trypsin or insulin affect either the levels or the ratios of the pyridine nucleotides. Most of this work has been carried out with mouse 3T3 fibroblasts and their SV40-virus transformed tumor cell counterpart. However, the results have been confirmed in 2 other cell lines, the NRK (normal rat kidney) cell line before and after transformation, and the L-929 line (mouse connective tissue cells).

Significance: The pyridine nucleotides (NAD^+ and NADH) are involved in many of the oxidation-reduction reactions of a cell. The ratio of NAD^+ to NADH is therefore a basic indicator of the metabolic state and growth properties of the cells. The differences in the ratio found between normal and tumor cells must therefore reflect the metabolic alteration in transformed cells. The ability to regulate this ratio in confluent transformed cells might thereby allow restoration to the growth and metabolic state of normal cells.

Proposed Course of Project: The project has been successfully completed.

Honors and Awards: None

Publications:

Schwartz, J.P., Johnson, G.S., Pastan, I. and Passonneau, J.V.: The effect of growth conditions on NAD^+ and NADH concentrations and the NAD^+/NADH ratio in normal and transformed fibroblasts. J. Biol. Chem. (in press).

Serial No. NDS(I)-70 LNNS/CN 1802
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of living nerve and muscle fibers by differential interference microscopy

Previous Serial Number: Same

Principal Investigator: Susan M. Billings, Ph.D.

Other Investigators: Henry deF. Webster, M.D.

Cooperating Units: None

Man Years:

Total:	0.
Professional:	0.
Other:	0.

Project Description:

Proposed Course of Project: This project has been terminated.

Serial No. NDS(I)-70 LNNS/CN 1803
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electron microscopic study of axon-Schwann cell relationships prior to myelination in peripheral nerves

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: John Martin, B.S.

Cooperating Units: None

Man Years:

Total:	0.
Professional:	0.
Other:	0.

Project Description:

Proposed Course of Project : This project has been terminated.

Serial No. NDS(I)-73 LNNS/CN 1995
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Morphological studies of CNS demyelination and remyelination in model systems

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Paul J. Reier, Ph.D.
Marian W. Kies, Ph.D.
Shirley H. Wray, M.D., Ph.D

Cooperating Units: Section on Myelin Chemistry, LCM, NIMH
Department of Neurology, Harvard Medical School

Man Years:

Total:	3.7
Professional:	1.9
Other:	1.8

Project Description:

Objectives: Several lines of evidence have suggested that the optic nerves of Xenopus tadpoles would be a useful model system of CNS myelin for studying the morphological parameters of demyelination and remyelination. Briefly, in these nerves the relationships of axons, myelin sheaths, and glial cells, their fine structure, and the morphological features of myelin formation all resemble those of mammalian experimental animals and humans. Xenopus tadpoles are transparent. Their optic nerves can be observed directly and it is relatively simple to transect them or expose them to injected materials without injuring adjacent vessels, nerves, or muscles. Also, recent observations have shown that when tadpoles are exposed to hexachlorophene, the myelin lesions are most severe in the optic nerves. The selective vulnerability of these CNS myelin sheaths to hexachlorophene suggested that they might be a useful model system for testing the demyelinating activity of other agents. Finally, if immature amphibian optic nerves are transected, they are known to be capable of regenerating and reestablishing functional retinotectal connections; the electron microscopic appearance of the regenerating axons and their subsequent remyelination has not been described. Therefore, to extend our observations on the potential usefulness of tadpole optic nerves as a model system for studying the mechanism of myelin formation, maintenance, breakdown, and regeneration, our

objectives were: (1) to improve currently available methods for surveying optic nerve myelin sheaths and studying the incidence of demyelinating lesions; (2) to start studying the demyelinating activity of sensitized lymphocytes from guinea pigs ill with experimental allergic encephalomyelitis (EAE); (3) to begin testing the demyelinating activity of sera from patients with demyelinating disease along with suitable control sera; and (4) to transect tadpole optic nerves and study both degenerative and regenerative phases with special attention to the morphological parameters of remyelination.

Methods Employed: (1) Whole mounts of aldehyde fixed tadpole optic nerves were prepared and examined with a differential interference microscope. For comparison, stained sections of other nerves that had been post-fixed, dehydrated, and embedded were studied by phase and electron microscopy. (2) Tadpoles were injected, either intraperitoneally or subcutaneously around one optic nerve, with lymphocyte suspensions from guinea pigs ill with EAE or appropriate control lymphocyte suspensions. At intervals after injection, tadpoles were fixed by aldehyde perfusion and their optic nerves were either prepared as whole mounts or they were post-fixed, dehydrated, embedded, sectioned, and stained for phase or electron microscopic examination. (3) Similar methods were used to process optic nerves from tadpoles that had received subcutaneous injections of human sera around one optic nerve. (4) In other tadpoles, one optic nerve was transected and a segment was removed. At intervals ranging from 4 hours to 3 months, the optic nerves were processed for microscopic examination so that the relationships of the region being studied and the transection site were known. Both whole mount preparations and extensive series of thick and thin serially mounted sections were available for study.

Major Findings: (1) Use of differential-interference contrast illumination enabled us to optically section whole mounts of tadpole optic nerves. Many myelin sheaths could be surveyed quickly over long distances at relatively high resolution. Lesions, consisting of focal areas of myelin breakdown and vacuolated ovoids in macrophages, could be identified easily and counted in these whole mounts. In sections studied by phase and electron microscopy, the appearance of normal myelin sheaths and the demyelinating lesions was similar. Transverse or longitudinal sections from several levels of each nerve took a long time to prepare and study; they did not provide reliable quantitative data on the incidence of demyelinating lesions. (2) In preliminary comparisons, tadpole optic nerves examined 7 days after intraperitoneal or subcutaneous injection of lymphocytes from guinea pigs ill with EAE contained significantly more demyelinating lesions than those exposed to lymphocytes from control guinea pigs. At later intervals after injection, the experimental optic nerves contained fewer lesions (incidence similar to controls), some hyperplastic astrocytes, and perhaps a few regenerating myelin sheaths. These experiments are currently being repeated and extended. (3) Only a few pairs of human sera (patients with demyelinating disease and controls) have been used in a small series of blind trials. The available preliminary evidence suggests that human sera can penetrate

into the optic nerves and produce demyelinating lesions. More tests are in progress. (4) In the distal stumps of transected optic nerves, axonal degeneration began within 16 hours; by 13 days, only centrally located astrocytes, processes with lipid debris, and a few oligodendrocytes were present. Regenerating axons were observed distally 19 days after transection. Throughout the regenerative period, the astrocyte was the main cell type observed. The astrocytes were generally clustered in the central region of the nerve parenchyma; their processes extended radially to the pial surface, forming longitudinal compartments for the regenerating axons. At 19 days, a few of the larger regenerating axons were partially surrounded by a thin collar of oligodendrocyte cytoplasm as remyelination began. At 34 days, large axons were more numerous and some were surrounded by compact myelin sheaths with up to 10 lamellae. Myelin spirals with 1-2 turns surrounded many other axons.

Significance to Biomedical Research and the Program of the Institute: These observations have demonstrated that Xenopus tadpole optic nerves are now a much more useful model system for studying many aspects of CNS myelin breakdown experimentally. A simple, quick procedure has been devised to survey individual CNS myelin sheaths within a CNS tract by sectioning them optically. Lesions can now be identified and counted without the time consuming effort of preparing and surveying large numbers of stained sections mounted on glass slides. We can now easily process the number of nerves required to test the demyelinating activity of lymphocytes and sera from patients with multiple sclerosis and other demyelinating diseases. Various CNS myelin constituents such as basic protein, galactosecerebroside, etc., can be used to prepare sensitized lymphocytes or immune sera for similar tests. Thus, we feel that the development of this whole mount technique may have provided investigators interested in multiple sclerosis and mechanisms of demyelination with an important new morphological tool. Most of our research effort is now invested in experiments to demonstrate its usefulness convincingly.

Model systems suitable for studying factors affecting the capacity of CNS axons and their myelin sheaths to regenerate after injury are also of major importance in paraplegia research programs. Tadpole optic nerves are almost the only heavily myelinated CNS tracts that can be easily transected under direct observation with insignificant injury to its blood supply and adjacent structures. The pace and morphological features of both axonal and myelin sheath regeneration have been characterized. They demonstrate that these nerves are also an excellent model system for testing the effects of hormones, drugs, and other substances on regeneration in CNS tracts. Such tests could also easily include physiological observations.

Proposed Course of Project: To be continued. Two manuscripts are in preparation.

Honors and Awards: None

Publications:

Webster, H. deF., Ulsamer, A. G., and O'Connell, M. F.: Hexachlorophene induced myelin lesions in the developing nervous system of Xenopus tadpoles: Morphological and biochemical observations. J. Neuropath. Exp. Neurol. 33: 144-163, 1974.

Serial No. NDS(I)-73 LNNS/CN 1996
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Myelin membrane structure - morphological comparisons in intact CNS tissue and subcellular brain fractions

Previous Serial Number: Same

Principal Investigator: Henry deF. Webster, M.D.

Other Investigators: Jean-Marie Matthieu, M.D.
Richard H. Quarles, Ph.D.

Cooperating Units: Developmental and Metabolic Neurology Branch, NINDS

Man Years:

Total:	0.2
Professional:	0.15
Other:	0.05

Project Description:

Objectives: (1) To study the electron microscopic appearance of rat CNS myelin fractions varying in glycoprotein content. (2) To compare, in Jimpy and control mice, the fine structure of optic nerve myelin sheaths with that of "myelin" fractions isolated from the CNS for biochemical study.

Methods Employed: Following aldehyde fixation, myelin fractions and optic nerves were postfixed in a solution of potassium ferrocyanide before dehydration and embedding. Sections of appropriate blocks were surveyed with the light microscope before cutting and staining thin sections for electron microscopic study.

Major Findings: (1) Compared to conventional preparative techniques, the above procedure improves the preservation of myelin. The intermediate line is double and the periodicity corresponds more closely to that observed in fresh myelin by X-ray diffraction. (2) Electron microscopic examination of subfractions of adult rat CNS myelin showed that loose membranes and vesicular profiles predominated in the glycoprotein containing heavy subfraction. Multi-layered membrane whorls with the lamellar structure of compact myelin were found in the light subfraction that had the highest content of basic protein. (3) In control and Jimpy littermates, an electron microscopic comparison of optic nerve myelinating axons and CNS myelin fractions showed the following: in the optic nerves of the 9 day old control mice, there were about 400 thinly myelinated fibers; by age 17 days, there

were more than 15,000 axons surrounded by much thicker myelin sheaths. At age 17 days, more than 90% of the axons in Jimpy optic nerves were still unmyelinated. About 200-500 relatively large axons were surrounded by myelin sheaths with 7-15 layers. The lamellar spacing in some resembled that found in control myelin sheaths but in others, the layered structure was less compact and the distance between the less dense lines was increased. Fractions of controls contained multi-layered whorls with the lamellar structure of myelin. They were not present in fractions isolated from Jimpy brains; instead, they contained 55 Å membrane profiles that were found singly or in loosely packed aggregates.

Significance to Biomedical Research and the Program of the Institute: (1-2) Morphological localization of the glycoprotein that has been found in myelin subfractions is of fundamental importance in that this component may have a major role in oligodendrocyte-axon interactions that influence myelin formation and maintenance. The above findings are consistent with the idea that glycoprotein is concentrated primarily in oligodendrocyte plasma membranes, loose myelin spirals, or very thin sheaths rather than in compact multi-layered myelin sheaths that are known to have a high content of basic proteins. (3) In Jimpy mice, fractions prepared by techniques to isolate myelin had neither biochemical nor electron microscopic features of myelin; a very severe myelination deficit was found also in the optic nerves and other CNS tracts. These findings add significantly to the evidence showing that the major glycoprotein found in fractions is closely associated with myelin *in situ* and has an important role in myelin formation.

Proposed Course of Project: These findings have been presented at the Annual Meeting of the American Society for Neurochemistry, March, 1974, and have been submitted for presentation at the VIII International Congress of Neuropathology, September, 1974. Other correlative morphological and biochemical studies of CNS myelin are in progress.

Honors and Awards: None

Publications:

Matthieu, J.-M., Quarles, R. H., Brady, R. O., and Webster, H. deF.: Variation of proteins, enzyme markers and gangliosides in myelin subfractions. Biochem. Biophys. Acta 329: 305-317, 1973.

Serial No. NDS(I)-74 LNNS/CN 2082
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cellular Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Human muscle spindles: Fine structure of the IA sensory endings

Previous Serial Number: None

Principal Investigator: William R. Kennedy, M.D.

Other Investigators: Henry deF. Webster, M.D.

Cooperating Units: University of Minnesota School of Medicine

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: To study the fine structure of the IA sensory endings of human muscle spindles and characterize their geometry, organelle content, and their surface membrane relationships with the spindles' muscle fibers.

Methods Employed: Muscle spindles were obtained by biopsy of the Extensor Indicis Proprius of human volunteers who were judged normal from the findings of neurological examinations, nerve conduction tests, electromyography, and muscle biopsy. The biopsies were immersed in glutaraldehyde and the spindles were isolated by gentle dissection before post-fixation, dehydration, and embedding. Other spindles were isolated and studied physiologically before fixation. Both transverse and serially mounted longitudinal sections of the spindles' equatorial regions that contained the IA sensory endings were studied with the light and electron microscopes.

Major Findings: The fine structure of spindles fixed before and after physiological study did not differ significantly. As the IA axon joined the intrafusal muscle fiber, it was covered only by basement membrane. The organelles in the endings were similar to those found in experimental animals and included neurofilaments, microtubules, agranular reticulum, vesicles, and mitochondria. In addition, each of the endings examined contained two arrangements of fine filaments, 75 Å in diameter, that have not been described previously. The first included long, ovoid masses of parallel, densely packed, thin filaments that were partially surrounded by mitochondria.

Usually, these aggregates were centrally located in the axon terminal and were parallel to its longitudinal axis. The second arrangement was a polygonal network of fine filaments located beneath the plasmalemma.

Significance to Biomedical Research and the Program of the Institute: A project goal of major importance has been achieved: namely, to show that the cellular preservation in human muscle spindles fixed during or after in vitro isolation and physiological study is not significantly different from that obtained in experimental animals by immediate in situ fixation. Now studies that correlate the fine structure and physiological activity of the human muscle spindle can be performed and the results compared with those obtained in spindles from patients with neuromuscular disease. Also, tests can be performed with drugs that may be useful in treating spasticity, a disability that occurs frequently in multiple sclerosis and other neurological diseases.

The fine filaments described above resemble the actin-like microfilaments that are found in a variety of cells. Present evidence suggests that they are concerned with cell movement and thus, it seems reasonable to think that the fine filaments described above are involved in adjusting the sensory ending's surface membrane to the shape changes that occur during stretch and relaxation of the spindle.

Proposed Course of the Project: Some of these observations have been presented at the American Neurological Association's annual meeting and will also be presented at the International Congress on Muscle Disease. A manuscript describing the fine filaments in the IA sensory endings has been accepted for publication in "Anatomical Record." A second manuscript is in preparation. The correlative studies noted above will be carried out by Dr. Kennedy and his collaborators at the University of Minnesota School of Medicine.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/CP 1997
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cerebrovascular reactions to ischemia in the Mongolian gerbil

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LNNS/CP 2104 and Serial No. NDS(I)-74 LNNS/CP 2105.

Serial No. NDS(I)-73 LNNS/CP 1998
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Central biogenic amines in edema and ischemia

Previous Serial Number: Same

Principal Investigator: Jonathan Costa, M.D., Ph.D.

Other Investigators: Umeo Ito, M.D.
Igor Klatzo, M.D.
Maria Spatz, M.D.

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years:

Total:	1.8
Professional:	1.8
Other:	0.0

Project Description:

Objectives: Several reports indicate that some biogenic amines may significantly affect the intensity of the brain tissue reaction to injury, and that 5-hydroxytryptamine may be an essential factor in the production of brain edema. This study was undertaken to explore this possibility, using as an experimental model brain edema produced by the application of a cold metal plate to the exposed cerebral cortex of the cat. The other part of our investigations refers to evaluation of some biogenic amines (serotonin, dopamine, and norepinephrine) in cerebral ischemia produced experimentally in gerbils by clipping the common carotid artery.

Methods Employed: Cold lesions were produced in cats by applying a cold (-50^{o}) metal plate to the exposed cerebral cortex for 30 seconds. At various times after the operation, the brain tissue containing the site of cortical injury, edematous white matter, as well as, more remote control areas was subjected to study by: (1) fluorescent microscopic observations according to the Falck technique, and (2) quantitative assays for 5-hydroxytryptamine using a modification of the method of Maickel. Since platelets seemed to be a potential source for the 5-hydroxytryptamine, the origin of this amine in cold lesions was ascertained by labeling platelet 5-hydroxytryptamine, producing cold lesions, and measuring the specific activity of 5-hydroxytryptamine in control and injured brain areas. Whole cat blood (10 ml preserved with USP Formula A citrate dextrose) was incubated with ^{3}H-5-hydroxytryptamine

creatimine sulphate (specific activity 7.3 Ci $mmole^{-1}$), in a final concentration of 1.37×10^{-6}M, for 30 minutes at 37°C. Labeled blood was reinjected into the cat and a lesion was made. Tissue 5-hydroxytryptamine was extracted into butanol and acidified heptane (after Maickel) and counted by liquid scintillation.

Cerebral ischemia can be produced in about 30% of Mongolian gerbils (Meriones unguiculatus) by a simple occlusion of the common carotid artery on the neck. The brain tissue from hemisphere affected by ischemia, from the opposite hemisphere, as well as, the brains of control gerbils were subjected to quantitative assays of serotonin, dopamine and norepinephrine after different periods of carotid occlusion.

Major Findings: In cats with cold injury the blood vessels confined to the area of injury showed a bright, yellowish-green fluorescence in the sections prepared by the Falck method for fluorescence microscopy. Fluorescence of this nature was conspicuous in the walls of arteries, veins, and capillaries. A few blood vessels showed an irregular, "mottled" pattern of yellowish-green fluorescence in their lumina. Otherwise, the thrombotic material filling numerous blood vessels revealed only a faint bluish autofluorescence. The characteristic yellowish-green fluorescence was not observed either in brain tissue adjacent to fluorescent vessels or in the blood vessels outside of the region of injury. Microspectrofluorometry of the yellowish-green fluorescent vessels in the tissue sections revealed that this fluorescence had the excitation-emission spectra and photodecomposition rate characteristic of 5-hydroxytryptamine.

Assaying the area of cortical injury and the edematous white using a modification of the method of Maickel demonstrated that 5-hydroxytryptamine in the injured cortical region was 329 $\pm$ 30% (mean $\pm$ s.e.m.) of that in the contralateral uninjured cortex; that in the underlying edematous white matter remained at control levels. In cats reinjected with labeled blood 5-hydroxytryptamine in the injured cortex had a specific activity 1500% of that in the control cortex and that in both edematous and control white matter. After intravenous injection of 100μCi of ^{3}H-5-hydroxytryptamine binoxalate, 5-hydroxytryptamine in the injured cortical region had a specific activity 800% of that in control cortex, edematous white matter and control white matter. The quantitative assays on ischemic gerbils revealed only the preliminary data indicating a reduction in levels of individual biogenic amines tested.

Significance to Biomedical Research and the Program of the Institute: The significance of 5-hydroxytryptamine accumulation in injured blood vessels remains to be ascertained. It is possible that this phenomenon may occur in vascular injuries other than those produced by cold, probably in those associated with thrombus formation. The possibility that 5-hydroxytryptamine acting on cerebral blood vessels may affect their permeability is supported by the recent observations from our laboratory that 5-hydroxytryptamine and other biogenic amines reaching arterioles and venules from the brain

parenchyma may alter their permeability to protein tracers. It is conceivable that the presence of appreciable amounts of 5-hydroxytryptamine in injured blood vessels may significantly affect the duration and intensity of vascular leakage, which is a crucial factor in the dynamics of vasogenic brain edema.

Proposed Course of the Project: The investigations on 5-hydroxytryptamine in injured blood vessels will proceed to correlate the appearance of this amine with the duration of increased permeability of these vessels. The study on biogenic amines in cerebral ischemia will be resumed with another principal investigator.

Honors and Awards: None

Publications: Costa, J. L., Ito, U., Spatz, M., Klatzo, I., and Demirjian, C.: 5-Hydroxytryptamine accumulation in cerebrovascular injury. Nature 248: 135-136, 1974.

Serial No. NDS(I)-74 LNNS/CP 2093
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of pressure gradients within brain tissue during the formation of vasogenic brain edema

Previous Serial Number: None

Principal Investigator: Hans J. Reulen, M.D.

Other Investigators: Igor Klatzo, M.D.
Robert S. Graham, B.S.

Cooperating Units: Department of Neurosurgery
University of Mainz, Mainz, West Germany

Man Years:

Total:	1.00
Professional:	.75
Other:	.25

Project Description:

Objectives: Experiments were performed in order to evaluate the concept that an increase in local interstitial fluid pressure may play a paramount role in the dynamics of the vasogenic type of edema.

Methods Employed: Assuming that the pressure measured by the cotton wick technique represents the hydrostatic interstitial tissue pressure, the tissue pressure was recorded with pressure transducers in the white matter adjacent and remote to a local cold injury in the opposite hemisphere and in the cisterna magna. The cold injury was inflicted to the right frontal pole in the anesthetized and artificially ventilated cats. Spreading of vasogenic edema was followed by intravenous injection of sodium fluorescein at the time of operation and of Evans Blue 3 hours later. Animals were sacrificed 6 hours following the injury. Water content was measured in consecutive blocks dissected from a horizontal tissue slice taken at the level of the tip of the wick.

Major Findings: Normal interstitial fluid pressure (IFP) ranged 1-4 mm Hg. below the CSF pressure and responded well to respiratory pulsations and changes in $paCO_2$. The development of vasogenic brain edema was associated with an increase in local IFP in edematous white matter adjacent to the

lesion and with the occurrence of a variety of pressure gradients within the tissue and the CSF. The pressures were always highest adjacent to the lesion and fell gradually toward the remote areas. At the time of sacrifice, pressure differences between the corresponding recording sites in the injured and uninjured hemispheres amounted to 8-13 mm Hg. Tissue pressures in the edema territory were significantly higher than the CSFP, while the pressure in the distant and opposite normal areas remained below the CSFP. Comparison of local IFP and local tissue water content revealed a close relationship. The shape of this curve shows a rapid IFP increase above CSFP with small increases of edema fluid. Thereafter, additional increases in edema fluid influences the tissue pressure to a lesser extent. Since the major part of this fluid accumulation has been shown to be extracellular, this relationship seems to represent the pressure/volume relationship of the extracellular space.

Significance to Biomedical Research and the Program of the Institute: These experiments allow a new insight into the dynamics of vasogenic type of brain edema. Our studies have shown previously that the hemodynamic pressure provides a primary force for spreading exsudated serum contents and edema fluid through the brain tissue. The movement of edema fluid encounters the resistance of the interstitial compartment and this results in elevation of the IFP in the area of edema. It is thus apparent that two major factors in dynamics of vasogenic edema must be systolic blood pressure and the IFP. It follows that reduction or elevation of the latter may greatly influence the dynamics of edema process. This work obviously contributes to basic understanding of brain edema.

Proposed Course of Project: The major effort will be directed now to study the dynamics of vasogenic brain edema under modified IFP conditions. This will be achieved by (a) changing the CSFP, and (b) changing the interstitial compartment by subjecting the animals to a generalized cytotoxic brain edema (by hexachlorophene intoxication). The findings from these studies are scheduled to be presented at the Second International Symposium on Intracranial Pressure, Lund, Sweden, June 1974.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNNS/CP 2104
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of the "no-reflow" (NR) phenomenon in Mongolian gerbils

Previous Serial Number: None and incorporating Serial No. NDS(I)-73 LNNS/CP 1997

Principal Investigator: Umeo Ito, M.D.

Other Investigators: Igor Klatzo, M.D.
Maria Spatz, M.D.
K. G. Go, M.D.

Cooperating Units: Department of Neurosurgery, University of Groningen
Groningen, The Netherlands

Man Years:

Total:	2.5
Professional:	1.6
Other :	.9

Project Description:

Objectives: The concept of "no-reflow" (NR) phenomenon was introduced by Ames et al. (1968) describing an impairment of cerebrovascular circulation in areas of the brain submitted to ischemia. Using carbon black perfusion shortly after release of an obstruction producing a total cerebral circulatory arrest, the authors described sometimes extensive areas of the brain in which the blood vessels failed to show filling with carbon particles. The present study was undertaken in view of an implication of potential clinical significance of NR phenomenon with regard to a possibility that such secondary impairment of cerebral circulation may be ultimately determining the severity and the extent of ischemic brain damage after release of an original arterial obstruction.

Methods Employed: The present investigation on the NR phenomenon was undertaken using Mongolian gerbils (Meriones unguiculatus) in which, due to anatomical irregularities of the circulus of Willis, an occlusion of the common carotid artery on the neck produces in about 30% of animals an ischemic injury in the ipsilateral cerebral hemisphere. In this model the possibility of dealing with larger series of experimental animals offers a

special advantage for comparing a number of various parameters involved. Mongolian gerbils weighing 60-80 gms. were anesthetized with sodium pentobarbital (5 m gm/100 gm). The left common carotid artery was exposed on the neck and clipped with the Heiffetz aneurismal clip for various periods of time. Gerbils with symptoms indicating a cerebral infarction according to Kahn's (1972) criteria were termed "sensitive," whereas, those which showed no symptoms after recovery from the anesthesia were called "insensitive." In some animals, EEG tracing was carried out in order to recognize "sensitive" animals at earlier stages during the anesthesia.

For evaluation of the NR phenomenon carbon black suspension (1 ml per animal) was injected slowly (during 30 sec.) into the femoral vein before the termination of an experiment at different time intervals after carotid clip release. Groups of gerbils were subjected to the left common carotid occlusion for 30 minutes, 1 hour, 3 hours and 6 hours respectively, each group being divided into subgroups corresponding to 30 seconds, 5 minutes, 10 minutes, and 15 minutes duration of the clip release before carbon perfusion.

The systemic arterial blood pressure was monitored throughout the experiments by intrafemoral artery catheter connected with transducer.

For assessment of an effect of sympathomimetics on NR phenomenon epinephrine, norepinephrine and isoproterenol respectively were used. The animals were injected intravenously with an individual sympathomimetic twice, once 30 seconds before clip release and the second time 2 minutes before the termination of the experiment.

Effect of pO_2 and pCO_2 on NR phenomenon was evaluated by exposing the animals to a constant flow of 20% O_2 or 10% CO_2 in a chamber for the last 30 minutes before the termination of an experiment. Blood samples for the blood gas analysis were collected from the animal's leg protruding from the plastic wall of the chamber.

The filling of the cerebral vessels with carbon black was evaluated either under the dissecting microscope following fixation by 10% formalin immersion of the brains or under the light microscope in sections stained with cresyl violet or hematoxylin and eosin.

The blood content of the cerebral vessels was outlined by Lepehne-Pickworth benzidine method demonstrating in black hemoglobin of the erythrocytes. The CBF was assessed by radioautographic method of Reivich using ^{14}C.

Major Findings: The NR phenomenon was recognizable by the presence of pale areas conspicuous on the greyish-black background of unaffected parts of the brain. These pale filling defects were confined, as a rule, to the hemisphere corresponding to the side of occlusion with exception of a few cases where, in addition some smaller filling defects were visible also in the other hemisphere.

The occurrence of the NR phenomenon in relation to the duration of carotid clipping and to the time interval following release of occlusion indicated that in gerbils subjected to different times of occlusion, no NR phenomenon could be demonstrated when the carbon black perfusion was performed later than 10 minutes following release of the clipping. Otherwise, the relationship between the NR phenomenon and the duration of occlusion was evident from the fact that after release of 30 sec. duration, a 100% incidence of NR was demonstrable in groups of 3 and 6 hours occlusion, whereas, after 30 sec. release the NR phenomenon was absent following 30 minutes occlusion and was present in one out of 5 animals in 1 hour occlusion group.

The recordings of the systemic blood pressure revealed a close relationship of incidence of the NR phenomenon to the drop in blood pressure which invariably followed the release of carotid clipping. It was evident that in animals with 6 hours occlusion a precipitous drop of blood pressure, recorded 30 seconds after release, corresponded to a 100% incidence of the NR phenomenon, whereas, 10 minutes after release, at which time NR could not be more demonstrated, the systemic blood pressure was well on the way to recovery.

Further evidence of dependence between the incidence of the NR phenomenon and the fall in the systemic blood pressure was provided by data on the effect of certain sympathomimetic substances. Epinephrine which was the most effective in preventing the drop in blood pressure following release of carotid occlusion was also most effective in prevention of the NR phenomenon.

Significance to Biomedical Research and the Program of the Institute: An implication of potential clinical significance of this study pertains to a possibility that such secondary impairment of cerebral circulation as presented by the NR phenomenon may be ultimately determining the severity and the extent of ischemic brain damage after release of an original arterial obstruction. Demonstration in this study that variations in systemic blood pressure play a significant part in occurrence of the NR phenomenon opens a possibility of using a pharmacological control of blood pressure as a preventive agent in clinical situations.

Proposed Course of Project: The major part of this project has been carried out. Further information will be required with regard to relationship of the NR phenomenon to the development of ischemic brain edema.

Honors and Awards: None

Publications:

Klatzo, I., Ito, U., Go, K. G., and Spatz, M.: Observations on experimental cerebral ischemia in Mongolian gerbils. In: The Proceedings of the International Symposium on the Pathology of Cerebral Microcirculation. Berlin, West Germany, Walter de Gruyter & Co., 1974, pp. 175-177.

Serial No. NDS(I)-74 LNNS/CP 2105
1. Neuropathology and Neuroanatomical Sciences
2. Section on Cerebrovascular Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Behavior of the blood-brain barrier and the development of edema in the cerebral ischemia

Previous Serial Number: None and Incorporating Serial No. NDS(I)-73 LNNS/CP 1997

Principal Investigator: Umeo Ito, M.D.

Other Investigators: K. G. Go, M.D.
Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: Department of Neurosurgery, University of Groningen, Groningen, The Netherlands

Man Years:

Total:	2.3
Professional:	1.7
Other:	0.6

Project Description:

Objectives: One of the complications of cerebral ischemia is the development of brain edema. Two main types of brain edema are the cytotoxic and vasogenic varieties which can be differentiated on the basis of the behavior of the blood-brain barrier (BBB). The objective of this study has been to elucidate the nature of ischemic brain edema by studying in a model of experimental cerebral ischemia the behavior of the BBB and the abnormal increase in brain tissue fluid, the essential criterion of any type of brain edema.

Methods Employed: Mongolian gerbils (Meriones unguiculatus) in which, due to anatomical irregularities of the circulus of Willis, an occlusion of the common carotid artery on the neck produces in about 30% of animals an ischemic injury in the ipsilateral cerebral hemisphere, were used as an experimental model for cerebral ischemia. Further details about this model are described in project NDS(I)-74 LNNS/CP 2104. In this project, the left common carotid artery was clipped on the neck for various periods of time.

For evaluation of the BBB Evans Blue dye was injected intravenously as a tracer. The systemic arterial blood pressure was monitored by intrafemoral catheter connected with transducer.

Abnormal increase of the brain tissue fluid was estimated by wet/dry weight ratios. After decapitation, the brains were taken out and the separated hemispheres were weighed (fresh weight). After drying at 98°C for six days the samples were weighed again (dry weight).

Histological changes were evaluated in brain tissue fixed by paraformaldehyde perfusion, embedded in the paraffin and stained with hematoxylin eosin and cresyl violet.

Major Findings: The estimation of wet/dry weight ratios revealed a progressive accumulation of the fluid in relation to the duration of carotid occlusion. Definite evidence of brain edema was apparent in animals sacrificed after 3 hours of carotid clipping which showed 7.34 $\pm$ 1.01 mean swelling percentage in the left hemisphere. In animals with 18 hours occlusion the swelling of the infarcted hemisphere amounted to 22%.

The incidence of the BBB damage, evident by blue staining of the brain tissue, was related to the duration of ischemia and to the duration of release following carotid occlusion. Thus, e.g., in gerbils subjected to 6 hours ischemia all animals showed the BBB damage one hour after release of occlusion. On the other hand, gerbils with one hour left carotid occlusion showed 100% incidence of the BBB damage only 20 hours following release of carotid clipping.

Raising the systemic arterial blood pressure accelerated significantly the BBB damage; e.g., a 100% incidence of the BBB damage was evident in gerbils with 147.8 mean arterial blood pressure (MABP) within 15 minutes after release, whereas, in animals with the normal MABP (72.4) such incidence was present only after 5 hours of release of carotid occlusion.

The histological changes characteristic of ischemic damage clearly preceded the BBB injury. They were first recognizable in animals sacrificed 30 minutes after carotid occlusion. They were progressively increasing during the period of release of carotid occlusion. This was in agreement with the observation that the histological picture of ischemic damage was similar in intensity in gerbils sacrificed immediately 6 hours after ischemia with that in animals with one hour occlusion and sacrificed 20 hours after release of the clipping.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature of ischemic brain edema is potentially of great value for the clinical management of stroke patients. The studies described above indicate that following ischemia there is a development of brain edema which is primarily of cytotoxic type. Only in later stages when there is damage to cerebral blood vessels and their increased permeability to

BBB tracers, a leakage of serum components is adding a vasogenic component to overall picture of brain edema. These findings might be of importance for designing proper treatment of patients suffering from cerebral ischemia.

Proposed Course of the Project: It is planned to collect further observations on relationships between ischemia, edema, and the BBB changes. It is also intended to extend this project by correlating this data with biochemical changes.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-64 LNNS/ENP 1066
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The specificity of neuronal changes in cerebral infarcts

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	0.6
Other:	1.4

Project Description:

Objectives: To ascertain the specificity of nerve cell changes in acutely infarcted brain tissue due to vascular occlusion.

Methods Employed: Histochemical method for demonstration of phospholipid.

The study of post-mortem autolysis by different histologic and histochemical methods.

Major Findings: (1) Neurons undergoing acute changes, ischemic neuronal changes, in infarcted brain tissue manifest affinity to hematein.

(2) Another cell change characterized by rapidly disintegrating cytoplasm is not associated with such affinity to hematein.

(3) This staining reaction, according to tests with normal tissue, is found to be non-specific.

(4) The neuronal changes during post-mortem autolysis do not mimic those in infarcts.

Significance: Since the affinity to hematein with Baker's phospholipid method is non-specific, an involvement of lipid or phospholipid metabolism in the cytoplasm of affected neurons can not be implicated. On the basis of these results, the following concept has been developed, namely that the morphologic manifestation of the ischemic neuronal change is the effect of a post-mortem trauma on a pathologically altered neuron during handling of the brain. The intermingled large neurons which do not stain with hematein probably represent the prototype of neuronal reaction to lack of oxygen or interrupted blood flow.

Post-mortem autolysis results in changes which, according to staining qualities with different methods, such as Baker's hematein, eosin, luxol fast blue, and azocarmine, differ from those of the ischemic neuron. Thus, autolysis can not be used as a procedure to reproduce ischemic changes in neurons.

Proposed Course of Project: To perform experiments under such conditions that affected neurons can be adequately fixed for microscopic examination.

To study post-mortem autolysis in tissues in which the homeostatic milieu has been changed without destruction of the tissue. Simultaneous investigations with histologic techniques and implantation experiments may open new avenues of preservation for the brain and spinal cord.

Honors and Awards: None

Publications:

Cammermeyer, J.: "Ischemic neuronal disease" of Spielmeyer. A reevaluation. Arch. Neurol. 29: 391-393, 1973.

Serial No. NDS(I)-67 LNNS/ENP 1449
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The effect of cortisone on the retrograde neuronal changes

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To determine what effect cortisone treatment may have on the acute reactive phase and the subsequent recovery phase of motor neurons following transection of their axons.

Methods Employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-gallocyanin method.

Major Findings: Preliminary studies have revealed that the acute peripheral chromatolysis is not influenced by cortisone treatment although the incidence of reactive mitosis is depressed. However, because of subtle differences in the neuronal reaction, new material has been prepared for supplementary studies.

Significance: In view of the frequent use of cortisone, which is known to impair the rate of regeneration of severed peripheral nerves, it is of importance to determine whether the nerve cell body is significantly affected. Until a meticulous survey of the histologic material has been completed, no conclusion about the effect of cortisone can be formulated.

Proposed Course of Project: Microscopic analysis of the experimental material which has been prepared.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/ENP 1676
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A comparative study of the area postrema

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

Objectives: (1) To determine the effect of chronic meningitis on the topography of the roof of the fourth ventricle, and (2) to determine the effect of chronic trypan blue treatment on the area postrema in cat and monkey.

Methods Employed: Light microscope techniques.

Major Findings: (1) Defects in the roof of the fourth ventricle vary with species.

(2) The sensitivity of cats and monkeys to apomorphine is depressed by chronic treatment with trypan blue; no histologic changes were detected in a preliminary study of serial sections of the brain stem.

Significance: (1) The validity of the statement that the formation of holes in the roof of the fourth ventricle is very marked in man suffering from chronic meningitis with hydrocephalus will be tested by study of the dog, which frequently suffers from severe hydrocephalus. The formation of holes in the roof of the fourth ventricle as a means to maintain adequate cerebrospinal circulation is still disputed; a study of dog material should clarify current uncertainties about cerebrospinal fluid physiology under pathologic conditions.

(2) Since in the normal animal, retching and vomiting are supposed to be induced by apomorphine via chemoreceptors in the intact area postrema, the fortuitous demonstration that cats treated with trypan blue have a lowered sensitivity to apomorphine could be indicative of damage to the area postrema. The absence of microscopically discernible changes may cast doubts on the theory that this region acts as a chemoreceptive area for the vomiting reaction; however, other histologic methods may shed new light on the mechanism involved in the present experimental model.

Proposed Course of Project: (1) Histologic preparation of brains from dogs suffering from hydrocephalus.

(2) Examination of material from cats chronically treated with trypan blue, with the aid of the fluorescence method on freeze-dried material, to determine whether under the given experimental conditions biologically active amines are affected.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/ENP 2002
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mast cells in the brain

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.1
Professional:	0.1
Other:	1.0

Project Description:

Objectives: To determine the role of mast cells in the central nervous system.

Methods Employed: Examination of microscopic sections from the brains of various animals with the aid of a specific staining technique for mast cells.

Major Findings: Mast cells occur in some species in practically all parts of the brain but with considerable differences between species, regions, the two sides of the brain and different age groups. The appearance of mast cells varies greatly; pallor, as an expression of loss of biologically active material, is often associated with loss of the nucleus. No association is found between distribution of mast cells and handedness, as demonstrated in swimming and feeding experiments with cats and monkeys.

Significance: Since mast cells contain a variety of substances - heparin, histamine, serotonin, dopamine, etc. - which are known to act on coagulability and viscosity of blood, diameter and permeability of blood vessels and neuronal function, and since these cells occur in many parts of the brain but in varying numbers, it is assumed that their presence depends on

functional requirements. If this is the case, they may play a significant role in blood flow and neuronal interaction in the brain. The microscopic demonstration of pallor and nuclear loss may suggest that mast cells disintegrate in situ as their functional capacity is exhausted.

Proposed Course of Project: To verify whether distribution of mast cells is associated with nervous functions, such as handedness, animals have been subjected to different tests. In view of the preliminary negative results, others, placed on different diets, are being studied histologically in order to determine whether the accumulation of mast cells in different regions can be influenced by metabolic factors.

The specificity of the histologic demonstration of nuclear loss is being investigated in serial sections of different animal species with a varying content of mast cells.

Honors and Awards: None

Publications:

Cammermeyer, J.: Mast cells and postnatal topographic anomalies in mammalian subfornical body and supraoptic crest. Z. Anat. Entwicklungsgesch. 140: 245-269, 1973.

Serial No. NDS(I)-73 LNNS/ENP 2003
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The thalamo-choroidal body

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To assess the ultramicroscopic structure of a region found to be prominent in the cat, and labeled thalamo-choroidal body.

Methods Employed: Various histologic techniques have been used to ascertain the type of constituting elements.

Major Findings: In the cat, a small region situated on either side of the roof over the third cerebral ventricle was found to have a remarkable structure, rich in blood vessels and collagenous fibers, nerve cells, supporting glial elements, plasma cells and mast cells.

Significance: The incidental observation of a special organ at sites of attachment of the roof covering the third ventricle is of interest because of its position near the choroid plexus, which is involved in cerebrospinal fluid circulation. It is speculated that such specialized structures as the thalamo-choroidal bodies serve a chemoreceptive or baroreceptive function for control of blood flow to the choroid plexus.

Proposed Course of Project: To elucidate the submicroscopic and histochemical composition of the thalamo-choroidal body with other techniques.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/ENP 2004
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The pathogenesis of myelopathies

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To assess the pathogenetic factors in spinal cord lesions.

Methods Employed: For an orientation of the characteristics of edema within the spinal cord, the volume of each segment of the spinal cord is measured prior to and after immersion in water for varying lengths of time.

Major Findings: (1) When the spinal cord is immersed in fluids, the degree of swelling varies with tonicity of the solute as well as with region.

(2) Splitting of the surrounding membranes demonstrated a far greater swelling which in the intact spinal cord is restricted by the membranes.

Significance: In some preliminary reviews, it was found that the human spinal cord becomes firmer during swelling because of the restrictive action of the superficial membranes, a reaction also noticeable in the cat spinal cord.

Since resistance to swelling by the unyielding membranes results in abnormal blood circulation, edema can cause spinal cord lesions. Knowledge of the role of edema in the pathogenesis of spinal cord diseases is important for the establishment of guidelines for treatment and prevention of spinal cord edema occurring as a complication to a number of human diseases, such as trauma, viral infection (poliomyelitis), allergic manifestations (multiple sclerosis), cancer, etc. The results of post-mortem experiments purporting to mimic intravital processes will serve as a foundation for additional experimental investigations.

Proposed Course of Project: To develop techniques to determine how blood flow is compromised.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/ENP 2005
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Heredopathia atactica polyneuritiformis (Refsum's disease)

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To summarize the pathologic changes in heredopathia atactica polyneuritiformis (Refsum's disease).

Methods Employed: Routine histopathologic techniques.

Major Findings: (1) Accumulation of fat in brain, liver and kidney; (2) atrophy with formation of "onion bulbs" in peripheral nerves and loss of motor nerve cells in spinal cord; (3) loss of myelin sheaths in certain fiber tracts in the medulla oblongata and the dorsal column of the spinal cord; and (4) loss of nerve cells in the inferior olivary nucleus.

Significance: In the first descriptions of the changes in the brain and the peripheral nerves, the principal investigator (Nord. Med. 29: 617, 1946; addendum in Refsum, Acta Psychiat. Neur., Suppl. 38: 1-303, 1946; J. Neuropath. Exp. Neur. 15: 340-361, 1956) predicted that the fundamental lesion was a defect in lipid metabolism, a suggestion which was confirmed in investigations by German biochemists, Klenk and Kahlke (Hoppe Selyers Z. Physiol. Chem. 333: 133-139, 1963) many years later.

From a review of 15 publications and of the microscopic material from 7 of these cases, it was concluded that (a) the deposition of fat in various parts is of secondary nature, associated with the level of fat or phytanic acid in the blood, (b) peripheral nerve lesions are non-specific, (c) changes in the brain can be prevented by dietary regimen, and (d) the metabolic abnormality resulting in excess phytanic acid in blood and organs does not seriously affect neurons.

Proposed Course of Project: New cases will be studied as they become available to determine factors contributing to individual differences in clinical and morphologic manifestations.

Nonors and Awards: None

Publications:

Cammermeyer, J.: Heredopathia atactica polyneuritiformis: Pathologic anatomy. In Vinken, P. J., and Bruyn, G. W. (eds.): Handbook of Clinical Neurology (in press).

Serial No. NDS(I)-65 LNNS/FN 1231
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Organization of synaptic connections in the mammalian brain

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Edward L. White, Ph.D.
Dennis M. D. Landis, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMDD

Man Years:

Total:	2.2
Professional:	1.8
Other:	0.4

Project Description:

Objectives: To map anatomical and functional relationships between synapses in the vertebrate central nervous system. Specific regions of the brain are selected for detailed analysis.

Methods Employed: Appropriately prepared brains are examined by electron microscopy. Three dimensional reconstructions based on electron microscopical examination of serial sections are correlated with the results from examining Golgi impregnated brains with the light and electron microscopes. Alternatively, known sources of synaptic input are destroyed and the degenerated synapses mapped at appropriate times after making the lesion. In the last year, two new techniques have been added to our approach. Leucine H_3 autoradiography has been used to identify the origins of synaptic terminals. The acquisition of a freeze-fracture apparatus has made possible examination of the membrane faces of synapses, an aspect not seen with conventional electron microscope sections.

Major Findings: The major new findings this year concern the genetic control of the development of certain synaptic patterns and differentiation of the postsynpatic membrane of excitatory and inhibitory synapses.

In Balb C mice a certain type of cortical synapse was found to be lacking even though the other three types of synapse in this region of the olfactory cortex were present in normal numbers. Furthermore, the normal

processes which would have produced the synapse were present so that the Balb C apparently lacks the genetic information to form or maintain a particular synapse. This would be the first demonstration that particular synaptic systems are under genetic control. The autoradiographic technique was used to investigate reinnervation of cells in the medial superior olive, a component of the ascending auditory system. It was surprising that no evidence of new synapse formation was found three months after destroying half of the synaptic input to these cells. Quantitative analyses with the electron microscope showed that the lack of new synapse formation was not due to disappearance of the synaptic sites due to shrinkage or due to occupation of these sites by other synapses.

The postsynaptic membranes of a variety of known excitatory and inhibitory synapses from olfactory bulb and cerebellum were examined in freeze-fracture faces. Excitatory synapses were found to have an array of particles embedded in the postsynaptic membrane coextensive with the synaptic junction. Similar particles were lacking or not specifically clustered at the synaptic junction of inhibitory synapses.

Significance to Biomedical Research and the Program of the Institute: Much effort in neurological research has been directed to mapping the axonal tracts connecting different areas of cerebral grey matter. Our effort is directed toward understanding what occurs at the termination of these tracts. We have shown previously that complex synaptic patterns exist in various regions of the brain each subserving specific types of physiological activity. This year we have expanded our consideration to genetic and environmental factors controlling development and maintenance of these synaptic patterns. The knowledge that genetic diseases could be expressed by specific changes in the organization of these systems is helpful in understanding the losses produced by these diseases. It is helpful in understanding the loss produced by brain injuries that some types of synapses apparently do not retain the ability to occupy synaptic sites vacated by damaged synapses. Also, knowledge about the types of synaptic interaction in different regions of the central neurons is helpful in understanding the sites of action of various neuropharmacological agents on the mammalian brain. It is particularly relevant in understanding the mechanisms of action of drugs on the nervous system to know the deployment of the various receptors for these drugs. There is reason to believe that our findings of different arrangements of postsynaptic membrane particles in different types of synapse is pertinent to this problem though more direct evidence about the nature of these membrane particles is needed.

Proposed Course of the Project: Dr. White has left and Dr. Landis will be leaving this year. A definitive publication on the lack of regeneration of synapses in the auditory system is being prepared; these data were presented at the annual meeting of the American Association of Anatomists. The work with Dr. Landis was presented at the annual meeting of the Society for Neuroscience and definitive publications have been accepted by the Journal of Comparative Neurology.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/FN 1442
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Permeability of cellular layers in the vertebrate nervous system

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Milton W. Brightman, Ph.D
Sachio Nabeshima, M.D.
Alan Shabo, M.D.
Dennis M. D. Landis, M.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMDD

Man Years:

Total:	1.3
Professional:	1.2
Other:	0.1

Project Description:

Objectives: Determination of the pathways taken by colloids and large molecular weight solutes to cross the various cellular layers associated with the nervous system. Currently being studied are: (1) ependyma covering the median eminence and area postrema; (2) arachnoid-dural membrane; (3) arachnoid villae; (4) canal of Schlemm in the primate eyes glial surface of the brain.

Methods Employed: The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase (MW 40,000) after it is injected into various brain or blood compartments of living, anesthetized mammals. The intercellular junctions fronting these compartments are studied with the electron microscope by the colloidal lanthanum or uranyl acetate methods. A new peroxidase of low molecular weight (microperoxidase MW k800) is now being used. The freeze-fracture technique has been used to see the internal structure of membranes.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the

astrocytic processes forming the limiting surface of the brain to reach the generally open interstitial spaces of the brain. However, peroxidase does not cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of "tight" junctions within the specialized epithelial cells lining the ventricular surface over these regions. The use of a smaller peroxidase has allowed it to be visualized crossing the capillary pores in the median eminence and area postrema.

A specialized layer of cells joined by tight junctions at the outer border of the arachnoid also prevents peroxidase from reaching the subdural space. Also, we have given our attention to the arachnoid villae where this arachnoid barrier layer becomes discontinuous at its apposition with the endothelium of the superior sagittal sinus. Study of sinus endothelium utilizing tilt microscopy and serial sections has revealed a system of minute pores which are responsible for bulb drainage of cerebrospinal fluid. We are now attempting to determine whether these pores might also be the basis for the valvular action of the arachnoid villae.

Another new ramification of our studies is to examine the meningeal barriers after acute infection with E coli; in these cases the tight junctions between barrier cells open and migration of leukocytes from the dura to the subarachnoid space occurs.

The site of drainage of the aqueous humor from the primate eye has also been examined and evidence produced that the endothelial vacuoles do not provide this channel as previously supposed, but instead it is provided by the endothelial intercellular clefts. The vacuoles have been demonstrated to be an artifact of fixation.

The membranes of astrocytotic processes facing blood vessels and cerebrospinal fluid have been found to have a peculiar internal structure characterized by the presence of assemblies of specialized subunits, presumably membrane proteins. This finding suggests that these glial membranes might have special permeabilities or pumping mechanisms which could be part of the blood-brain barrier.

Significance to Biomedical Research and the Program of the Institute: We are determining the permeability to a small protein of the various cellular layers associated with the mammalian nervous system. Our determinations are by means of cytological techniques that show specifically which components of which layers are permeable. Thus we are able to determine the cells as well as the probable mechanisms that control the chemical environment of the brain (blood-brain barrier and blood-cerebrospinal fluid barriers, blood aqueous barriers) in a normally functioning brain and eye. The new findings on the arachnoid villae give us specific knowledge about the mechanism of and control over drainage of cerebrospinal fluid. We are beginning to use our knowledge

of these normal mechanisms as a basis for studying the changes which occur in pathological conditions such as acute infections of the meninges. Demonstration that the intercellular junctions are the primary site of drainage of aqueous humor from the eye focus attention on these structures rather than the endothelial vacuoles as the probable site of disorder in glaucoma. Of particular interest is the conclusion suggested by the freeze-fracture study of glial membranes where they face intracerebral fluid compartments, that the glial may have some role in the blood-brain barrier.

Proposed Course of the Project: The work on the arachnoid has been presented to the American Association of Anatomists and the American Association of Neuropathologists. Much of this work is being prepared for publication; some will be incorporated into a Physiological Review which two of the authors are preparing. Dr. Nabeshima has left NIH and reports on his work are being prepared. Future work will concentrate on the normal and diseased arachnoid membrane and villae and on the structure of membranes in the barrier systems of the brain as revealed by freeze-fracture.

Honors and Awards: None

Publications:

Shabo, A. L., Reese, T. S. and Gaasterland, D.: Postmortem formation of giant endothelial vacuoles is Schlemm's canal of the monkey. Amer. J. Opthal. 76: 896-905, 1973.

Landis, D. M. D. and Reese, T. S.: Arrays of particles in freeze-fractured astrocytic membranes. J. Cell Biol. 60: 316-320, 1974.

Serial No. NDS(I)-69 LNNS/FN 1684
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structure and function of close intercellular (gap) junctions

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To study the structure and distribution of gap junctions and to show directly with the electron microscope their permeability to inter and intracellular tracers.

Methods Employed: Peroxidases of various molecular weights are applied to or injected into cells joined by gap or tight junctions and the peroxidase is subsequently localized with the electron microscope. We have elected to work on the crayfish because it has giant nerve cells joined by gap junctions which permit this experiment to be done with simple injection techniques and without damaging the nerve cells.

Major Findings: During this year we concentrated our efforts on two peroxidases, MW 1200 and MW 42,000, which represent the extreme limits in the range of sizes available to us. We injected these peroxidases after, as well as before, fixation and found that the gap junctions are impermeable to both tracers prior to fixation, but permeable after fixation. Thus the size of the cell-to-cell bridges associated with these gap junctions is considerably smaller in life than was expected. This finding, that the size of the intercellular channels in gap junctions increases during fixation, was confirmed this year using fluorescene labeled albumin.

Significance to Biomedical Research and the Program of the Institute: Intercellular electrical coupling has recently been shown to be important at

a variety of normal cellular appositions. Changes in this type of coupling appear to be a component of cancerous changes. Our study is an attempt to provide a morphological basis for electrical intercellular coupling by: 1. defining whether this communication occurs directly or via the intercellular spaces; 2. defining what size materials can pass from one cell to adjacent cells; 3. demonstrating directly the cell-to-cell channels that have been thought to be present within gap junctions. Our work over the last year has succeeded in putting an upper limit on the molecular weight of substances that can cross from one cell to another via the gap junctions we have studied, and has showed that these channels either undergo rearrangement of their structure or are supplemented by other channels during fixation for electron microscopy.

Proposed Course of Project: This project has been held in abeyance this year. Preliminary results were presented at the annual meeting of the American Association of Anatomists in April, 1971 and will be presented at the Federation Meetings. New findings were presented at the 1972 meeting of the Society for General Physiology and a report is in preparation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-71 LNNS/FN 1881
1. Neuropathology and Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structural basis of synaptic transmission

Previous Serial Number: Same

Principal Investigator: Thomas S. Reese, M.D.

Other Investigators: John E. Heuser, M.D.
Avery D. Nelson, Ph.D.
Maryanna Henkart, Ph.D.
Dennis M. D. Landis

Cooperating Units: Behavioral Biology Branch, NICHD and Laboratory of Experimental Pathology, NIAMDD

Man Years:

Total:	3.2
Professional:	2.7
Other:	0.5

Project Description:

Objectives: To determine the structural basis of transmitter release from synapses.

Methods Employed: (1) Frog sartorius nerve-muscle preparations are exposed to altered ionic environments or stimulated with various programs, and their responses are monitored by standard electrophysiological means until they reach a desired functional state. Then they are immediately fixed and prepared for examination with the electron microscope. Similar experiments are performed in solutions containing horseradish peroxidase and the horseradish peroxidase subsequently localized with the electron microscope. (2) Similar experiments were performed on preganglionic autonomic synapses in the frog. Crayfish stretch receptor synapses were examined in an attempt to find a favorable preparation to study inhibitory synapses. Most recently, the neuromuscular junction and frog sympathetic ganglion preparations were examined with the freeze-fracture technique.

Major Findings: The traditional fine-structural appearance of the synapse represents a resting or equilibrium state. With intense electrical stimulation and transmitter release approaching exhaustion, the intracellular organelles of the presynaptic terminal undergo well-defined, reversible

changes. These include mitochondrial swelling and migration to new regions, depletion of synaptic vesicles with coincident enlargement of the presynaptic membrane surface, and finally formation of coated vesicles and Golgi-apparatus-like bodies, apparently as intermediate states in the recycling of membrane back into synaptic vesicles.

When similar experiments are done in solutions containing horseradish peroxidase, the tracer progressively fills various membrane forms inside the synapse, thus tracing directly the recycling of membrane from coated vesicles to synaptic vesicles and back to surface membrane concomitant with transmitter release.

Identical results were obtained with autonomic synapses from the sympathetic ganglion indicating that new synaptic vesicles here are also formed by recycling of membranes. Examination of the inhibitory synapses on crayfish stretch receptor cells revealed that these synapses receive synaptic input from other sources. An incidental result of work on nueromuscular synapses in the rat is that RNA synthesis which accompanies overload hypertrophy is largely the result of proliferation of connective tissue cells.

Application of the freeze-fracture technique to automatic and motor synapses has shown that the only synaptic vesicles which are releasable are those attached to the presynaptic "fuzz". It is likely that these vesicles contain the readily releasable pool of transmitter.

Significance to Biomedical Research and the Program of the Institute: This work contributes to an understanding of the role of membranes in the storage and release of transmitter at synapses and shows that the mechanism for release of neurotransmitters is similar to that employed for release of glandular secretions. Also this work helps to broaden the general anatomical criteria for identifying synapses, thereby aiding future attempts to define the existence and location of functional contacts in the CNS, and most important, permitting attempts to define their physiological state in normal and pathological brain samples. Thus, it should be possible to distinguish normally active from pathologically active synapses in epileptogenic foci. The discovery that the RNA synthesis in muscle hypertrophy is the result of proliferation of connective tissue cells casts doubt on the idea that muscle cells proliferate in hypertrophy.

Proposed Course of Project: The work on transmitter release at the neuromuscular synapse has been completed and presented at several scientific meetings. It has also appeared as a full length paper. The work on the sympathetic ganglion is being presented at meetings this spring and will be completed within the next year. Drs. Heuser and Henkart have left NIH and their aspects of the work terminated; Dr. Landis will leave this year. The future course of this project will be to complete the work on the autonomic synapses with the freeze-fracture technique.

Honors and Awards: None

Publications:

Costa, J. L. and Reese, T. S.: Serotonin storage in platelets: estimation of storage-packet size. Science 183: 537-538, 1974.

Heuser, J. E., Reese, T. S. and Landis, D. M. D.: Functional changes in frog neuromuscular junctions studied with freeze-fracture. J. Neurocytol. 30: 109-131, 1974.

Heuser, J. E. and Reese, T. S.: Morphology of synaptic vesicle discharge and reformation at the frog neuromuscular junction. In Bennett, M. V. L. (Ed.): The Synapse, New York, Raven Press. In press.

Serial No. NDS(I)-71 LNNS/NC 1879
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemical changes in brain of transplacentally methylazoxymethanol (MAM) induced microencephaly in rats

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Gert L. Laqueur, M.D.
Grazyna Szumanska, Ph.D.

Cooperating Units: Laboratory of Experimental Pathology, NIAMD

Man Years:

Total:	1.50
Professional:	.75
Other:	.75

Project Description:

Objectives: The transplacentally MAM induced microencephaly in rats was reported to be associated with decreased intellectual capacity and the development of gliomas in 10% of the affected animals (Spatz, M. and Laqueur, G., Proc. Soc. Exp. Biol. Med. 129: 705, 1968; and Haddad, R., Rabe, A., Laqueur, G., Spatz, M., and Valsamis, M., Science, 163: 88-90, 1969). In the latest studies concerned with the lipid composition of microencephalic brains, a disturbance in myelin formation was found in these brains. Furthermore, the diminished amount of total lipids and phospholipids observed in microencephaly was due to reduction of phosphatidyl ethanolamine. The present investigation is a continuation of this study in order to explore the carbohydrate compartment of the MAMA processed brains.

Methods Employed: The microencephaly was induced by simple intraperitoneal injection of 20 mg/kg of MAMA on the fifteenth day of gestation. The control animals were treated similarly but received saline instead of MAMA. The experimental and control rats were sacrificed at 7, 14, 28, 60, and 90 days of age. Fresh frozen sections were used for the histochemical analyses of phosphylase, UDPG transferase, G-6-P dehydrogenase, and lactate dehydrogenase but for glycogen visualizations were perfused with paraformaldehyde.

Major Findings: Increased activity of brain phosphorylase a, b, and total (a and b) was found in the developing and mature cerebrum, cerebellum,

and spinal cord when compared to that of controls. The increase in the activity of phosphorylase b and total (a and b) was more prominent than that of phosphorylase a. The distribution of glycogen was the same in the experimental as in the control rats except for microencephalic cortex where an accumulation of fine glycogen droplets was seen in the first cellular layer. These changes suggest an altered carbohydrate metabolism. In addition, a decreased activity of glucose-6 phosphate dehydrogenase was seen in the cerebral cortex and medulla oblongata in microencephalic, when compared to control rats, indicating the involvement of the pentose cycle in the previously described disturbance of lipid metabolism.

Significance to Biomedical Research and the Program of the Institute: These investigations might be helpful in the understanding of some neurological conditions in which (1) abnormal synthesis or (2) degeneration of myelin takes place. Therefore, this or a similar model system might be useful for comparative studies of abnormal myelin formation observed in (1) malnutrition and (2) help in elucidating demyelinizing processes which may play a role, for example, in multiple sclerosis.

Proposed Course of Project: This project has been terminated.

Honors and Awards: None

Publications:

Laqueur, G. L. and Spatz, M.: Transplacental induction of tumors and malformations in rats with cycasin and methylazoxymethanol. Transplacental Carcinogenesis, 59-64, 1973.

Serial No. NDS(I)-71 LNNS/NC 1880
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Mayland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Hyperosmolar and $HgCl_2$ effect on the brain uptake of ^{14}C glucose analogues

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: Igor Klatzo, M.D.
Zbigniew M. Rap, M.D.
Stanley I. Rapoport, M.D.

Cooperating Units: Polish Academy of Sciences, Warsaw, Poland
Laboratory of Neurophysiology, NIMH

Man Years:

Total:	1.37
Professional:	1.12
Other :	.25

Project Description:

Objectives: In previous studies, we demonstrated an increased facilitated transport of glucose analogues from blood to brain following intracarotid perfusion of hypertonic solutions. This study was a continuation of the same project in order to (1) elucidate the relationship of the increased brain uptake of glucose to the condition of the blood flow in the cerebral hemisphere, and (2) to study the effect of $HgCl_2$ BBB injury on the brain uptake of glucose analogues.

Methods Employed: Young adult rabbits were used for these experiments. An intracarotid cannula was inserted in one of the common carotid arteries, the external carotid artery was ligated in order to perfuse the hypertonic urea or $HgCl_2$ directly into the internal carotid artery. The concentration of the urea perfusate was two or three molar and the concentrations of $HgCl_2$ was .01 mM or .08 mM.

For the estimating of blood flow, ^{14}C antipyrine was injected into the femoral vein ten minutes after hypertonic urea perfusion. The animals were sacrificed by decapitation and the section of brain tissue frozen quickly and subsequently cut in a cryostat and processed for radioautography.

The uptake of ^{14}C 2-deoxy-D-glucose into the brain was analyzed 10 minutes after $HgCl_2$ perfusion by using a modified double isotope technique of Oldendorf. The self-inhibition of ^{14}C 2-deoxy-D-glucose was determined with simultaneous injection of cold 2-deoxy-D-glucose with the labeled tracer. The brain uptake index was calculated per 100 mg of brain tissue.

$$BUI = \frac{\text{Tissue } ^{14}C \text{ / Tissue } ^{3}H}{\text{Injectate } ^{14}C \text{ / Injectate } ^{14}C} \times 100$$

Major Findings: The ^{14}C antipyrine radioautography of the brains perfused with hypertonic urea revealed an equal distribution of ^{14}C antipyrine in both the perfused and unperfused cerebral hemisphere. Thus, no direct relationship of the increased BUI for ^{14}C glucose analogues to an altered blood flow could be established by this method.

Intracarotid perfusions of .01 mM $HgCl_2$ decreased BUI for ^{14}C 2-deoxy-D-glucose to the level of maximally inhibited controls injected with cold 2-deoxy-D-glucose probably due to inhibition of the facilitated transport of ^{14}C 2-deoxy-D-glucose into the brain (BUI 16.3 $\pm$ .05 S.E., N=4, 14.35 $\pm$ 1.85 S.E., N=5, respectively). Passive diffusion of ^{14}C 2-deoxy-D-glucose occurred also with $HgCl_2$ perfusate of high concentration (.08 mM) although the BUI was below (26.8 $\pm$ 3.06 S.E., N=5) the one in controls (48.83 $\pm$ .83 S.E., N=10).

Significance to Biomedical Research and the Program of the Institute: The investigation of glucose transport across an altered blood brain barrier (BBB) such as following hyperosmotic and metabolic injury is of great interest and importance from the pathophysiological point of view of the BBB. (A) Since glucose is one of the most significant nutrients of the brain, the broadening of the knowledge concerning its passage from blood to brain in health and disease is of major importance. (B) It may help in elucidating the pathogenesis of many neurological disorders. (C) It may offer an understanding and a possibility to select the best therapeutic approach to given disease.

Proposed Course of Project: This model may be useful in the investigation of hypertonic effects on amino acids and biogenic amines transport from blood to brain.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/NC 1999
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Brain uptake of ^{14}C 3-O-methyl-D-glucose and ^{14}C sucrose in ischemic gerbils

Previous Serial Number: Same

Principal Investigator: Maria Spatz, M.D.

Other Investigators: I. Klatzo, M.D.
K. G. Go, M.D.

Cooperating Units: None

Man Years:

Total:	1.25
Professional:	.75
Other:	.50

Project Description:

Objectives: The Mongolian gerbils possess a high susceptibility for the development of cerebral infarction following unilateral ligation of the common carotid artery due to frequent absence of arterial communication between the cerebral and vertebral system (Levine, S. and Payan, H., Exp. Neurol. 16: 255-262, 1966, and Kahn, K., Neurol. 22: 510-515, 1972). The project described here was designed to evaluate the effect of ischemia on glucose transport from blood to brain as one of the multidisciplinary approaches for the pathophysiological investigations of cerebrovascular disorders. Recently we demonstrated an increased brain uptake of ^{14}C 2-deoxy-D-glucose after carotid arterial occlusion for various periods of time. This augmented ^{14}C 2-deoxy-D-glucose brain uptake was self-inhibited with cold 2-deoxy-D-glucose in all animals except for gerbils with cerebral symptoms subjected to 18 hours of carotid artery occlusion. The present investigation is a continuation study of ischemic effect on the transport of glucose and sucrose into the brain.

Methods Employed: Several groups of gerbils were subjected to left common carotid artery clipping for various periods of time and the release of the clip for five minutes. Thereafter, a double isotope technique was used for measuring the differential uptake of tracers injected as a mixture intracarotidally 15 seconds prior to decapitation. In this method $^{3}H_2O$ was

used as a reference with which the uptake of ^{14}C labeled 3-O-methyl-D-glucose or ^{14}C sucrose was compared. For the inhibition studies the injectate contained 2 mM phlorizin (cold) with the labeled 3-O-methyl-D-glucose. The brain uptake index (BUI) for the test substance was calculated as follows :

$$BUI = \frac{\text{Tissue } ^{14}C \text{ / Tissue } ^{3}H}{\text{Injectate } ^{14}C \text{ / Injectate } ^{3}H} \times 100$$

Major Findings: An increased brain uptake of ^{14}C 3-O-methyl-D-glucose was demonstrated in both sensitive (with cerebral damage) and nonsensitive (without cerebral damage) gerbils after 3, 6, and 18 hours of common carotid artery occlusion (BUI 65.47 $\pm$ 6.19 S.E., 60.75 $\pm$ 2.73 S.E., 66.84 $\pm$ 2.51 S.E. respectively, controls 43.71 $\pm$ 1.09 S.E.). In controls and ischemic gerbils cross inhibition of ^{14}C 3-O-methyl-D-glucose was obtained with simultaneous injection of unlabeled 2 mM phlorizin. A difference in the degree of inhibition was found between the sensitive and nonsensitive gerbils following 18 hours of carotid artery occlusion. The 3-O-methyl-D-glucose brain uptake was 43.77 $\pm$ 5.21 S.E., (N=5) in the sensitive, 24.31 $\pm$ .81 S.E., (N=6) in the nonsensitive, and 25.91 $\pm$ 1.42 S.E., (N=5) in control animals.

The BUI for ^{14}C sucrose was the same in the ischemic (BUI 7.5 $\pm$ .32 S.E., N=10) as in the control gerbils (BUI 7.72 $\pm$ .52 S.E., N=5) except for 18 hours ischemic sensitive gerbils which showed an increase in ^{14}C sucrose brain uptake (BUI 19.76 $\pm$ 3.9 S.E., N=3).

The cross inhibition of the increased ^{14}C 3-O-methyl-D-glucose brain uptake of the brain non-metabolizable hexose support our previous contention that the transfer of glucose analogues took place by facilitated transport in the sensitive and nonsensitive gerbils except for 18 hours sensitive (with cerebral damage) animals in which passive diffusion occurred, too. This assumption is supported by the normal ^{14}C sucrose passage at 6 hours and increased passage of ^{14}C sucrose at 18 hours after carotid artery occlusion (sensitive gerbils).

Significance to Biomedical Research and the Program of the Institute: The basic comprehension of the blood-brain barrier (BBB) functions concerned with the transport of nutritional substances from blood to brain following cerebral ischemia is of major importance (1) for the understanding of the pathophysiological process occurring in cerebrovascular disease and many other neurological disorders, and (2) for selecting the best therapeutic approach to a given disease.

Proposed Course of Project: This model system will be useful in studying the effect of ischemia on the transport of amino acids and biogenic amines.

Honors and Awards: None

Publications:

Spatz, M., Go, K. G., and Klatzo, I.: The effect of ischemia on the brain uptake of ^{14}C glucose analogues and ^{14}C sucrose. In: The Proceedings of the International Symposium on the Pathology of Cerebral Microcirculation. Berlin, West Germany, Walter de Gruyter & Co., 1974, pp. 189-193.

Serial No. NDS(I)-73 LNNS/NC 2000
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Brain edema in cerebral ischemia of gerbils

Previous Serial Number: Same

Principal Investigator: K. Gwan Go, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
Hanna Pappius, Ph.D.

Cooperating Units: Montreal Neurological Institute

Man Years:

Total:	.87
Professional:	.75
Other:	.12

Project Description:

Objectives: In human cerebral ischemia, brain edema is considered an important factor in causing mortality (Shaw, C., Alvord, E. and Berry, R., Arch. Neurol. 1: 161-177, 1959). Experimentally, cerebral ischemia can be easily produced in Mongolian gerbils by ligation of a single common carotid artery (Levine, S. and Payan, H., Exp. Neurol. 16: 252-255, 1966; Kahn, K., Arch. Path. 69: 544-553, 1972; and Ito, U., Go, K. G., Klatzo, I. and Spatz, M., American Association of Neuropathologists Meeting, 1973). Therefore, the gerbils were chosen as an animal model system for the investigation of brain edema in ischemia.

In our recent studies of ischemic brain edema in gerbils, a decrease in the percent of dry weight (i.e., an increased water content) was found in the cerebral hemisphere ipsilateral to the carotid artery occlusion as compared to the controls. The present investigation has been a continuation of this study to determine the electrolyte concentration in the brain in order to correlate the changes of the electrolyte to the water content of the brain in cerebral ischemia.

Methods Employed: Several groups of adult gerbils were subjected to unilateral clipping of the left common carotid artery for various periods of time (1, 3, 6, and 18 hours), and release for 5 or 60 minutes. For this study, only the gerbils with definite ischemic cerebral symptoms

(established clinically or by EEG) were selected for the determination of the brain water and electrolyte content. The brains from the decapitated animals were removed quickly and the separated hemispheres weighed immediately (fresh weight). Following drying at 98°C for six days, the samples were weighed again (dry weight) and used for the analyses of Na and K content by flame photometry.

Major Findings: The effect of the unilateral common carotid artery occlusion on the cerebral Na and K content was seen already after one hour of arterial clipping. There was a net loss of potassium (34 meg/kg dry weight) and a net gain of sodium (46 meg/kg dry weight) in the affected, as compared to the unaffected and the control cerebral hemispheres. These changes progressed (as was reported in the brain water content) with the length of the carotid artery occlusion. There were no significant differences observed in the Na and K concentration of the affected hemispheres whether the release period lasted 5 or 60 minutes after carotid artery occlusion.

Significance to Biomedical Research and the Program of the Institute: Cerebral edema occurs as one of the major complications of many neurological disorders such as ischemia, trauma, tumors, chemical poisoning, and others. The basic understanding of the type of edema and its development is very crucial for the clinician who is faced not only with the diagnosis, but the appropriate selection of treatment. Thus, various investigations of this problem are essential for finding the factor or factors responsible for the occurrence of cerebral edema and its treatment.

Proposed Course of Project: The study of brain edema in ischemia will be concerned with the continuous effort to differentiate the early cytogenic from the secondary vasogenic component of the cerebral edema. The investigation will include electron microscopic and radioisotopic evaluation of the ischemic brain in gerbils.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LNNS/NC 2001
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The effect of oxygen saturation and pCO_2 on glucose transport in the rabbit brain

Previous Serial Number: Same

Principal Investigator: Frank Berson, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	1.50
Professional:	1.25
Other:	.25

Project Description:

Objectives: Previous studies have shown that both hyperosmotic blood-brain barrier injury and ischemia cause an increase in the facilitated transfer of glucose from blood to brain. The purpose of this investigation was to evaluate the effect of oxygen saturation and carbon dioxide tension on the transport of glucose into the rabbit brain.

Methods Employed: Young adult rabbits were anesthetized and ventilation controlled with a small animal respirator. By varying the rate of ventilation and concentrations of inspired gases, it was possible to regulate the pO_2, pCO_2 and pH of arterial blood. Blood pressure was monitored throughout the experiments and blood gas levels measured in a pH/blood gas analyzer. A radioactive mixture of tritiated H_2O and a carbon-14 labeled glucose analogue was injected into the cerebral circulation via the right common carotid artery 15 seconds prior to decapitation. The brain was removed and a portion of cortex processed by standard techniques for radioactive counting. Uptake of the test substance was expressed in terms of a brain uptake index (BUI).

Major Findings: Abrupt lowering of oxygen saturation to below 18% resulted in a 40-45% decrease in the BUI of both 2-deoxy-D-glucose and 3-O-methyl-D-glucose. Superimposing severe hypercapnia on hypoxia had no additional effect. Mild to moderate hypoxia and hyperoxia at one atmosphere

did not affect glucose uptake. Increase in arterial pCO_2 with respiratory acidosis resulted in a decrease in BUI comparable for both analogues. Hypocapnia with respiratory alkalosis caused an increase in the BUI of 3-O-methyl-D-glucose. Moreover, there was an increased sensitivity to inhibition of ^{14}C labeled 3-O-methyl-D-glucose with cold (unlabeled) 3-O-methyl-D-glucose when compared with controls (Km = 0.5 mM and 5.0 mM, respectively).

Significance to Biomedical Research and the Program of the Institute : Oxygen saturation, pCO_2 and pH represent factors which, individually or combined, may play a role in altering the blood-brain barrier and contribute to the tissue damage resulting from cerebral ischemia. Since glucose is the basic cerebral nutrient, elucidation of factors which affect its uptake by the brain should help in understanding the pathophysiologic process involved in ischemia and may suggest means of preventing irreversible damage.

Proposed Course of Project: This model will be used to study the transport of amino acids into the brain. Also, blood flow studies using a xenon clearance method are planned in order to quantitate any effect the cerebral blood flow may have on uptake of glucose analogues under these particular experimental conditions.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNNS/NC 2083
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Glucose transport in cerebellar cultures

Previous Serial Number: None

Principal Investigator: Krystyna Renkawek, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
Margaret Murray, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.50
Professional:	1.50
Other:	0.

Project Description:

Objectives: The glucose transport from blood to brain was found to be increased following experimental ischemia in gerbils and decreased in acute hypoxia and hypercapnia in rabbits (Spatz, M., Go, K. G., and Klatzo, I., The Proceedings of the International Symposium on the Pathology of Cerebral Microcirculation, 1974 and Go, K. G., Berson, F., Klatzo, I. and Spatz, M., American Association of Neuropathologists meeting, 1973). The changes of brain uptake in a disease process may depend on the integrity of (1) the cerebral vascular bed and/or (2) some of the cellular elements as for example, astrocytes. Thus, these experiments were designed to utilize tissue culture for the study of transport phenomena across the cellular membrane elements in the brain under normal and pathological conditions.

Methods Employed: The Maximow technique was used for the cultures of newborn rats' cerebellum. Two cerebellar explants were placed on a collagen-coated coverslip, washed, and fed twice weekly. The glucose uptake was investigated with the nonmetabolizable hexose, ^{3}H-3-0-methyl-D-glucose in 14 day old cultures. The concentration of the tritiated tracer was ^{14}C/1 ml of nutrient media without glucose supplement. Groups of 6-7 sister cultures were washed three times with balanced salt solution (BSS) and incubated with .05 ml of nutrient media containing ^{3}H-3-0-methyl-D-glucose (.5 µc) with and without addition of 1 mM unlabeled tracer for 30 and 60 minute periods.

Thereafter, the medium was removed, saved, and the cultures washed three times with BSS. The pooled samples of tissue culture, removed medium and washing were weighed, solubilized and processed for liquid scintillation counting. Similar procedures were used for the evaluation of glucose uptake in the cerebellar cultures by radioautography.

Major Findings: This project is in a preliminary stage, since (without technical help) it took some time to standardize the proper nutrient requirements for the growth of cerebellar cultures without fresh human placental serum and to develop a suitable technique for the study of glucose transport

The cerebellar tissue cultures took up approximately 10% of the introduced ^{3}H-3-O-methyl-D-glucose at 30 minutes and 20-30% of the fed isotope at 60 minutes of incubation. The uptake of the glucose was considerably lower in the cultures incubated with the mixture of labeled and unlabeled tracer than with only labeled hexose containing medium at pH 7.2 (DPM/mg 2352.9 and 7365.9 respectively). The self inhibition of ^{3}H-3-O-methyl-D-glucose uptake suggest that the cerebellar tissue culture uptake of the inert hexose occurred by facilitated transport. This phenomena was absent when the pH of the nutrient media was above 7.2.

The appropriate method for the visualization of the glucose uptake in the cerebellar cultures by radioautography has been in the developing stage so far.

Significance to Biomedical Research and the Program of the Institute: The cerebellar tissue culture is an excellent model for the study of transport across the cellular membrane elements without the interference of cerebral vessels. These investigations have been focused on creating the environmental and cytogenic conditions in tissue cultures similar to the one existing in normal and diseased brain in order to determine the cellular functions in many neurological disorders associated with ischemia, edema, neurotoxicity and others.

Proposed Course of Project: The immediate investigations have been concerned with defining and eliciting the factors responsible for the glucose transport under normal conditions. Therefore, the relationship of the glucose uptake to the duration of the incubation, the kinetic and the influence of K and insulin on the glucose transport will be determined in these cultures. Thereafter, this model will be used to study the glucose transport under pathological conditions. Furthermore, amino acid transport will be investigated too.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNNS/NC 2084
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The growth and morphologic properties of cerebellar and meningeal capillaries

Previous Serial Number: None

Principal Investigator: Krystyna Renkawek, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
Margaret Murray, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.

Project Description:

Objectives: The regulation of nutrient substance movement from blood to brain have been attributed to reside in the cerebral capillaries (Krogh, A., Proc. Roy. Soc. (Biol.) 133: 140-200, 1946). However, until now none of the experimental models dealt with the preparation of the cerebral capillaries separate from other cellular elements of the brain. Therefore, the purpose of this study has been to establish capillary cultures from (1) cerebellum, and (2) from meninges and then investigate the function of endothelial cells.

Methods Employed: The Maximow technique was used for cultivation of capillaries from cerebellar or meningeal explants. The cerebellum was stripped from meninges and each one sectioned into small fragments. The cerebellar tissue was placed while the meninges were spread in collagen-coated coverslips. The cultures were fed and washed twice weekly. The nutrient medium was composed of equal parts of balanced salt solution (BSS), fetal calf serum, some serum ultrafiltrate supplemented with 300 mg% of glucose. The outgrowth of cells and the formation of capillaries have been observed by light and phase microscopy for 14-28 days. Thereafter, the cultures were fixed in solution and stained with azan and hematoxylin.

Major Findings: This project is in a very preliminary stage. So far it has been possible to observe an outgrowth of endothelial cells from the

cerebellar and meningeal explants. The endothelial cells appeared to grow in parallel and/or crossed chains of cells and forming small loops and were easily distinguished by form and pattern from other cells.

Significance to Biomedical Research and the Program of the Institute: The cerebellar and/or meningeal cultures when standardized will be an excellent model for studying the function of endothelial cells and their role in transport and/or metabolism of the brain in health and disease. Such information is of great importance for the understanding of many neurological disease processes, especially the cerebrovascular disorders.

Proposed Course of Project: At the present time, the studies are concerned with standardizing the growth of the cerebellar and/or meningeal capillaries and evaluate their histological and histochemical characteristics. Thereafter, several groups of capillary cultures will be utilized for the isotope studies of glucose, amino acids, and biogenic amine transport by autoradiography and liquid scintillation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNNS/NC 2085
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytobiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ultrastructural changes in cerebral ischemia of gerbils

Previous Serial Number: None

Principal Investigator: Erik Westergaard, M.D.

Other Investigators: Maria Spatz, M.D.
Igor Klatzo, M.D.
K. G. Go, M.D.

Cooperating Units: Anatomy Department, University of Copenhagen
Department of Neurosurgery, University of Groningen

Man Years:

Total:	1.75
Professional:	1.00
Other:	.75

Project Description:

Objectives: Cerebral infarctions occur in 30-50% of gerbils subjected to the occlusion of a single common carotid artery (Ito, U., Go, K. G., Klatzo, I. and Spatz, M., American Association of Neuropathologists Meeting, 1973). In the animals with cerebral injury, an augmented facilitated transport of glucose analogues was observed after six hours while increase passive diffusion of glucose occurred after eighteen hours of carotid artery clipping. In gerbils without apparent cerebral damage, only the facilitated transport of glucose analogues was found to be increased irrespective of the duration of carotid artery occlusion. These findings suggested that the observed change and type of glucose transport may depend on the integrity of the cerebral capillaries (Spatz, M., Go, K. G., and Klatzo, I., The Proceedings of the International Symposium on the Pathology of Cerebral Microcirculation, 1974). Thus, the present investigation was designed to evaluate the status of the cerebral parenchyma and the cerebral capillaries by electron microscopy in ischemic gerbils.

Methods Employed: Several groups of gerbils submitted to various periods of left common carotid artery occlusion and one hour release were injected with horseradish peroxidase (10 mg/100 g body weight) as the blood-brain barrier (BBB) tracer. The horseradish peroxidase was allowed to circulate

for five minutes and thereafter the brains were perfused with paraformaldehyde. Selective tissue sections of the cerebral hemisphere were processed for light and electron microscopy from animals with and without cerebral symptoms.

Major Findings: This project was started a month ago and is still in very preliminary stages. So far the brain section screened by light microscopy revealed changes in the gerbils with cerebral symptoms only. Capillary vesicular transport appears to be increased in the cerebral hemisphere ipsilateral to the common carotid artery occlusion for three and six hours while cerebrovascular lesions were seen in the 18 hour ischemic brains.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the nature and sequence of vascular and parenchymatous ischemic brain injury is of major importance in the understanding of the cerebrovascular disease process. The exploration of the established animal model system for cerebral ischemia should be helpful and useful in the comprehension and treatment of this disease process in man.

Proposed Course of Project: This investigation has been and will be concerned with the study of ultrastructural changes in the brain during and following different periods of cerebral ischemia in order to delineate the sequential pathological processes occurring in the cerebral vessels and parenchyma.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/NC 1443
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Lise Prescott, M.D.
Thomas S. Reese, M.D.
Stanley I. Rapoport, M.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH

Man Years:

Total:	0.9
Professional:	0.6
Other:	0.3

Project Description:

Objectives: To ascertain the effects of hypertonic salt solutions on the structure of tight junctions as they relate to the blood-brain barrier to exogenous peroxidase.

Methods Employed: Hypertonic (3.0 M) solutions of urea are applied topically to the cerebral cortex of rabbits or injected into the carotid artery. Trypan blue and horseradish peroxidase (HRP) are injected into the peripheral circulation just before the urea is administered. The effects of urea are being examined in replicas of frozen-cleaved tight junctions. Since fracture planes through endothelial tight junctions are very uncommon, replicas of choroid plexus and cultured kidney-derived cells, with numerous, long tight junctions will be treated with threshold and denaturing concentrations of urea.

Major Findings: In cerebral capillaries 90 seconds after the intracarotid injection of 3 M urea, trypan blue-albumin and peroxidase pass through tight junctions into the extracellular clefts between adjacent junctions. These interjunctional clefts are normally inaccessible to protein. Once having penetrated the junctions the peroxidase rapidly floods the extracellular clefts of the neuropil. In regions such as the median eminence that

lie outside the blood-brain barrier, adjacent ependymal cells are united not only by gap junctions but by tight junctions as well. In freeze-fracture, the gap junctions are discoid and highly ordered whereas in neighboring ependyma they form triangular patches. The rows of ridges comprising the tight junctions in the median eminence are fewer than the well developed ones of the choroid plexus but are sufficiently extensive to keep peroxidase from passing between ventricular and intercellular fluid.

Significance to Biomedical Research and the Program of the Institute: Threshold concentrations (2.5-3.0 M) of urea open endothelial tight junctions without damaging capillary cells or parenchymal cells. Therapeutic agents, normally excluded from the extracellular fluid of the brain by these tight junctions, can thus be introduced into the brain substance without damage to the capillaries or parenchymal cells.

Proposed Course of Project: In order to find ways of further modifying tight junctions in a reversible fashion, replicas of frozen-cleaved tight junctions immersed in 3 M urea compared to denaturing concentrations of 8 M urea will be compared. By such means, we can learn to recognize subtle changes in macromolecular structure of membranes at tight junctions and at gap junctions as well.

Honors and Awards: None

Publications: Brightman, M. W., Hori, M., Rapoport, S. I., Reese, T.S. and Westergaard, E.: Osmotic opening of tight junctions in cerebral endothelium. J. Comp. Neurol. 152: 317-326, 1973.

Serial No. NDS(I)-68 LNNS/NC 1587
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A blood-brain barrier to peroxidase in the normal and injured brain of elasmobranchs

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Thomas S. Reese, M.D.
Lise Prescott, M.D.
Igor Klatzo, M.D.

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: The results of the first phase have been completed and published, but it now must be made certain that the perivascular glia is the barrier by perfusing peroxidase retrogradely, i.e. from the cerebral ventricle.

Methods Employed: Exogenous horseradish peroxidase (HRP) is to be perfused ventriculo-cisternally in sharks and dogfish and its cytological distribution ascertained electronmicroscopically.

Major Findings: In two poorly-fixed specimens, HRP reached the perivascular basement membrane from the cerebral ventricles.

Significance to Biomedical Research and the Program of the Institute: If HRP reaches the basement membrane in well-fixed specimens, then the glial junctions are not barriers after all, or are one-way valve-like barriers, or the basement-membrane is the barrier.

Proposed Course of Project: To procure healthy specimens and repeat the ventricular perfusions to determine the differential roles of basement membrane and glial junctions as extracellular barriers to protein passage.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1678
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Distribution of exogenous proteins in brain tumors

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman, Ph.D.

Other Investigators: Lise Prescott, M.D.

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	1.1
Other:	0.3

Project Description:

Objectives: To determine differences in permeability of the endothelium in neoplasms to peroxidase and the availability of the extracellular spaces (ecs) of the tumors to this protein.

Methods Employed: Rous sarcoma virus (RSV) or Simian virus 40 (SV_{40}) are inoculated into the brains of newborn hamsters and, after the appearance of ataxia, circling or somnolence, peroxidase is injected intravascularly and allowed to circulate for 2 to 60 minutes. Thin sections of the tumor are examined electron-microscopically.

Major Findings: Many endothelial junctions in the choroid plexus papilloma induced by the SV_{40} virus are open and allow the passage of peroxidase through them. Some of the junctions, however, are tight and probably zonular. Many of the tumor vessels are fenestrated, the fenestrae outnumbering the junctions by at least four to one. Thus, many but not all of the tumor vessels are permeable to protein because of their patent junctions and, perhaps, because of their fenestrae. Instead of astrocytic end-feet, the vessels are ensheathed by tumor cells which are united by discontinuous tight junctions which would impede but not prevent extracellular movement of protein.

Significance to Biomedical Research and the Program of the Institute: The open endothelial junctions and, presumably, the more numerous fenestrae

underlie the heightened permeability of tumor vessels to circulating protein. The concurrence of tight junctions that are continuous in some tumor vessels and of discontinuous but extensive tight junctions between some tumor cells could account for the uneven penetration of radio-opaque tracers and, perhaps, of therapeutic agents into the extracellular spaces of comparable brain tumors.

Proposed Course of Project: The work is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1805
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structural changes within the membranes of stimulated smooth muscle and neurosecretory cells

Previous Serial Number: Same

Principal Investigator: Lise Prescott, M.D.

Other Investigators: Milton W. Brightman, Ph.D.
Harold Gainer, Ph.D.
Jeffery L. Barker, M.D.

Cooperating Units: Behavioral Biology Branch, NICHD

Man Years:

Total:	0.9
Professional:	0.6
Other:	0.3

Project Description:

Objectives: To examine the alterations in membrane structure that accompany KCl stimulation of smooth muscle and neurosecretory cells in order to understand the mechanism of vesicle fusion with the cell membrane.

Methods Employed: The marine gastropod, Aplysia, has an abdominal ganglion surrounded by a sheath containing many rows of isolated smooth muscle cells which, in some regions are intermingled with neurosecretory axons. Both muscle and neurosecretory cells are superficial and easily reached by fluid bathing the sheath. Thus, by merely excising the ganglion and connectives, the whole preparation may be stimulated by KCl solutions without the need for dissection. Such thin specimens are also readily frozen without prior fixation or cryo-protection. The ganglion and sheath are immersed in 120mM KCl for 15 to 60 minutes, fixed, glycerinated and frozen-cleaved. The fractured surfaces inside the cell membranes are then replicated with platinum.

Major Findings: As in mammalian smooth muscle cells, the Aplysia sarcolemma is indented in the form of many pits or caveolae, whose function is unknown. These experiments have provided the first demonstration that smooth muscle cell membrane responds to elevated extracellular KCl by forming new

fusion sites between pits and cell membrane. These pits are distinguished from the membranes of neurosecretory granules and from endothelial pits of mammalian capillaries in having coarsely granular membrane faces. There is no discernible change in number of fusion sites between neurosecretory granules and cell membrane. In the smooth muscle, the pits are on the same membrane with peculiar grills and ridges that may be related to hemi-desmosomes. Pits, grills and ridges are stable. either reducing the pH of the fixative to 4 or omitting fixation and glycerination has any effect on them whereas the other particles in the membrane faces become reordered.

Significance to Biomedical Research and the Program of the Institute: Extracellular KCl, when elevated, causes the sarcolemma to form new pits, thereby increasing its surface area. These pits might be endocytotic but, more likely, take the place of a transverse tubule system normally absent from vertebrate and molluscan smooth muscle. The pits may thus be involved in the coupling of excitation with muscle contraction.

Proposed Course of Project: To determine whether or not the pits are pinocytotic, ganglia have been immersed in peroxidase solutions before and during stimulation. Mammalian myoepithelial cells and intestinal smooth muscle cells will be exposed to KCl in order to see whether the increase in pitting is a widespread effect.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LNNS/NC 1965
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The effect of biogenic amines on blood-brain barrier to peroxidase

Previous Serial Number: Same

Principal Investigator: Erik Westergaard, M.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.2
Other:	0.3

Project Description :

Objectives: To determine the effects of biogenic amines on the permeability of cerebral vessels to exogenous peroxidase after first establishing the degree of permeability in normal vessels.

Methods Employed: Intravascular injection of horseradish peroxidase (HRP) followed by ventriculocisternal perfusion of amines and electron-microscopic evaluation of HRP distribution.

Major Findings: In normal, untreated mice, rats and hamsters given dialyzed peroxidase or ferritin intravenously, certain pial and parenchymal arterioles transfer the proteins in vesicles to the perivascular fluid. The endothelium of muscle-coated vessels thus differs from capillaries in the central nervous system by having numerous membrane pits and vesicles capable of transporting protein from blood to the extracellular spaces of the brain parenchyma and to the subarachnoid space. When high concentration (50 to 800 μg) of serotonin or nor-adrenalin are perfused ventriculo-cisternally, this transfer appears to be augmented.

Significance to Biomedical Research and the Program of the Institute: This unexpected transfer of protein in normal animals probably accounts for the small but finite amount of albumin and globulin normally present in the cerebrospinal fluid of mammals, including man. These sites of transfer could

be routes for antigens or immune globulins involved in auto-immune reactions of the central nervous system.

Proposed Course of Project: Further experiments to corroborate the enhancing effect of biogenic amines on protein transfer will be carried out.

Honors and Awards: None

Publications: Westergaard, E. and Brightman, M. W.: Transport of proteins across normal cerebral arterioles. J. Comp. Neurol. 152: 17-44, 1973.

Serial No. NDS(I)-74 LNNS/NC 2086
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regeneration in vertebrate and invertebrate nerves

Previous Serial Number: None

Principal Investigator: Richard Shivers, Ph.D.

Other Investigators: Milton W. Brightman, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.2
Other:	0.3

Project Description:

Objectives: To examine structural changes inside the membranes of nerves and surrounding glial cells during degeneration and regeneration.

Methods Employed: The roots of the 6th ganglion in crayfish contain both motor and sensory nerves that are situated just above the ventral body wall; since these nerves are so superficial only little dissection is required and, therefore, scarring is minimal. One to five weeks after sectioning, the proximal nerve stumps are fixed, frozen, cleaved and replicated. In post-weanling rats, the hypoglossal nerves are sectioned and 48 hours to 6 months later, processed like the crustacean nerves.

Major Findings: The axons of the crayfish nerves are normally wrapped concentrically in multiple glial and connective tissue sheets. This arrangement is lost during regeneration. The glial cells become disorganized but maintain their close spatial relationship to regenerating axons. The glial membrane shows two striking changes:

(a) the number of gap junction increases but the size of each junction is so diminished that only a few large intramembranous particles comprise a single junction
(b) there is a proliferation of small pits or bosses, depending on the membrane face viewed. These small loci are arranged in regular rows within the glial cell membrane.

Significance to Biomedical Research and the Program of the Institute: The intramembranous changes point to an active response of the glial cell during nerve regeneration. Since the distal stump of the nerves in crayfish does not degenerate for many months, it is highly unlikely that the loci are phagocytic. They are too small and there is little or no debris to be imbibed. The glial cell may, at the same time, be forming and reforming myriad small junctions during nerve and glial growth. Gap junctions have been implicated in metabolic exchange between epithelial cells and the many small junctions here may likewise be involved in nerve-glial interchanges.

Proposed Course of Project: To further study this interrelationship and to compare these events with those within the membranes of regenerating hyposlossal nerve in mammals.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Neural Control, Intramural Research
National Institute of Neurological Diseases and Stroke

Karl Frank, Ph.D., Chief

During Fiscal Year 1974 the Laboratory of Neural Control has continued its efforts: 1. to develop further insights into the basic mechanisms by which control of motor output is effected in the central nervous system; 2. the development of techniques for making direct contact with the nervous system for the purpose of transferring information directly into or out of it; 3. the exploration of practical areas of application of basic knowledge about the nervous system to the relief of the neurologically handicapped, and 4. the development and promulgation of an awareness of the social consequences of applying the rapidly emerging new and powerful techniques of neural control.

Before summarizing the year's accomplishments of the Laboratory of Neural Control, it is appropriate to list some statistics describing the Laboratory. Space assigned continues at 3800 square feet on the third and fifth floors of Building 36 on the NIH campus.

The staff of LNLC numbers 17. Eight have permanent Civil Service or permanent Commissioned Corps appointments. The others either have limited tenure appointments or are in the first year of a Career Conditional Civil Service appointment. There are two Visiting Fellows, two Research Associates, one Stay-in-School and four persons unofficially associated with the Laboratory.

Motor Control Systems Operating in the Spinal Cord

Basic research has continued concerning intrinsic properties of alpha motoneurons and the physiological, morphological and histochemical characteristics of the muscle fibers innervated by them. A major objective is the determination of the patterns of usage of motor units of different types during a variety of movements in the freely moving animal. An important aspect of this basic research is the organization of synaptic input to alpha motoneurons from segmental reflex and descending systems leading to the control of motor behavior.

Compensatory hypertrophy has been studied in the gastrocnemius muscle of the cat. No change in percent composition of different types of motor units was found despite a significant increase in maximal tetanic tension production.

Techniques for Making Connections with the Nervous System

Development of techniques for transfer of information into the nervous system (inward information transfer) or techniques for recording the temporospatial activity of selected neurons for use as control signals (outward information transfer) are essential for all applications of neural control. Electrical stimulation of nerve cells and recording of the electrical signals of their activity continue to be the methods of choice for inward and outward information transfer. However, the possibility of using local chemical excitation or inhibition of the nervous system is not excluded.

In the investigation of materials suitable for histological implantation, iridium has been added to the list and evaluation of cyanoacrylate adhesives, gold, tantalum, epoxy, Parylene C, Teflon and certain medical grade silastics has continued.

The newly developed "map pin" electrode continues to be the most promising technique for chronic recording of single spike potentials. Removal of Parylene C insulation from the tip of the electrode is accomplished by exposing it to a high AC voltage arc. Single cell signals have been recorded for eight months in cats and monkeys. One of the major concerns with this technique is the possibility of long-term enzymatic attack of the insulation on the electrodes.

Contractors in the Neural Prosthesis Program are carrying out chronic tests of the new capacitor electrodes developed by LNLC. A commercial supplier has been established to meet the increasing demand for these electrodes which provide safe stimulation of the nervous system.

One of the most important technical advances sponsored by LNLC has been the development of a micromanipulator for introducing microelectrodes into the human brain. Pulsation of the brain during neurosurgical procedures has prevented sustained recording from single nerve cells prior to the development of the present device which uses air bearings and a sensor to follow the movement of the cortex, thus minimizing the relative movement between the electrode and the nerve cell whose action potentials it is recording. Trials on monkeys indicate that the device is ready for use on humans.

Groove electrode arrays for chronic recording from nerve fibers have been tested in cats and rabbits and may permit chronic recording of single fiber action potentials in active unanesthetized animals.

A "cable and barb" multipolar electrode is being investigated for single motor unit recording in muscle. This technique

shows promise but may require sophisticated data analysis techniques to improve signal-to-noise ratios.

A variation of the successful free-floating "map pin" electrode developed by LNLC has been used for recording single units in the dorsal root ganglion in cats. This technique may permit chronic recording of single unit afferent activity in active unanesthetized animals. This would be a major technological breakthrough.

Progress has been made in development of an implantable, remotely controlled, fixed-dose injector which will release a 5-50 microliter dose of any drug into a distant tissue space.

Cortical Mechanisms of Voluntary Motor Control

The aim of this project is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor response measurements. In addition, it is important to determine how much control is possible over the firing patterns of cortical neurons. If it can be shown that an individual can control the firing patterns of specific neurons, the chances of building a centrally controlled prosthesis will be markedly improved.

Input-Output Pathways of Dorsal Root Ganglion Cells

Classically sensory nerve fibers enter the spinal cord through the dorsal roots and have their cell bodies in the dorsal root ganglia. Most physiological experiments investigating spinal cord mechanisms are based on this assumption. However, there is some evidence that there are ganglion cells scattered in the ventral roots and that a large proportion of unmyelinated fibers in the ventral roots have their cell bodies in the dorsal root ganglion. This study, just beginning, is designed to explore the existence and proportion of such aberrant spinal nerve fibers.

Neural Prosthesis Program

This program is entering its third year. It is a major effort of the LNLC directed toward the application of recent advances in basic neurosciences, engineering and physical sciences to the development of prostheses for the neurologically handicapped.

The current objectives of the program are to determine the feasibility of a sensory prosthesis for the blind utilizing direct electrical stimulation of the visual cortex, a bladder prosthesis for patients with neurogenic bladders utilizing

electrical stimulation of the sacral spinal cord, functional neuromuscular stimulation with intramuscular electrodes in patients with upper motor neuron lesions and evaluation of the effects of chronic cerebellar stimulation for relief of medically intractable epilepsy. The feasibility studies are being carried out primarily through contracts with eight institutions. These contracts are budgeted and administrated mainly through C&FR. Project Officers and intramural collaboration are within LNLC-IR.

Functional Neuromuscular Stimulation

Case Western Reserve University has implanted intramuscular electrodes in paralyzed patients and is studying the degree of proportional control that can be achieved. Sequential stimulation through multiple electrodes is utilized to provide smooth muscle contractions and to reduce fatigue. They are also studying the effects of electrical stimulation on the physiological and histological properties of muscles in both normal and spinal animals.

A forearm orthosis using sequential electrical stimulation through three electrodes placed in the flexor digitorum superficialis and profundus of a C-5 quadriplegic patient was evaluated. Utilizing a proportional output shoulder position transducer to control frequency of stimulation, the patient could independently grasp and use objects such as pencils, cups, combs and eating utensils.

Continuous electrical stimulation for periods as short as 12 days is sufficient to increase the proportion of the fatigue resistant fibers of cat tibialis anterior.

Bladder Evacuation

Both Duke University and the University of California at San Francisco are stimulating the spinal cords of both normal and spinal animals to determine if cell populations exist in the cord which can be selectively stimulated to cause bladder detrusor contraction with simultaneous sphincter relaxation.

Active bladder contraction is easily achieved during electrical stimulation of the sacral spinal cord in dogs. However, no region of the spinal cord has been found which when stimulated results in relaxation of the striated urethral sphincter.

Sensory Prosthesis

The University of Rochester, using sighted and blinded rhesus monkeys, is studying methods of optimizing information input to the visual cortex, survival of electrical excitability during long-term stimulation, and the effects of blindness on these factors.

The Massachusetts General Hospital is investigating the relationships between stimulus parameters and afterdischarges following electrical stimulation of the visual cortex in animals. They are also studying the effects of changes in electrode parameters and electrode size by stimulating visual cortex of human subjects who require occipital craniotomies.

No practical level of electrical stimulation of the visual cortex in macaques has been found which does not produce at least a temporary increase in threshold for detection by the animal.

At threshold currents for producing an afterdischarge with cortical surface stimulation in the cat, the afterdischarge involves a region of the brain around the stimulating electrode with a radius of approximately 2 mm. With suprathreshold stimulation, this radius can increase to at least 4 mm.

The Huntington Institute of Applied Medical Research is concentrating on developing methods of confining current spread during electrical stimulation. They are also continuing their series of chronically stimulated animals with subsequent histopathological examination of surrounding tissue.

Thin, unreinforced silastic electrode arrays have developed a curling distortion resulting in cortical compression after implantation on the surface of cat cortex. Histologically, neuronal displacement occurs with little or no neuronal loss.

Basic studies at the Environmental Impact Center are determining the electrochemical reactions at electrode-electrolyte junctions during the passage of current and methods of identifying and minimizing possible toxic byproducts.

During multiple, symmetrical, biphasic, constant current pulsing into simulated cerebrospinal fluid, the potential of a platinum electrode tends to oscillate around the hydrogen potential. Electrode corrosion as indicated by increases in real surface area has also been noted.

The University of Florida is performing long-term *in vitro* tests of potential electrode materials using scanning electron microscopy and atomic absorption spectrophotometry to evaluate corrosion of these electrodes during electrical current passage. Promising materials are used as chronic stimulating electrodes in animals with subsequent histopathological study of surrounding tissues.

Platinum-10% iridium and gold when used as materials for stimulating electrodes show severe pitting and crevice corrosion. Titanium oxide and tantalum pentoxide have shown excellent

corrosion resistance, most likely due to their passive oxide surfaces.

Cerebellar Studies

A request for proposals has been released for a new contract to study the effects of cerebellar stimulation for prevention or termination of seizures in nonhuman primate models of epilepsy.

The Project Officers have coordinated some of their research activities at NIH with those of the contractors and have organized a workshop, now held annually on neural prostheses.

Fundamental to all the neural prostheses currently under study or anticipated in the future is the development of safe, effective means of selectively exciting or inhibiting small populations of neurons.

Serial No. NDS(I)-69 LNLC/OC 1686
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Motor Control Systems in the Spinal Cord

Previous Serial Number: Same

Principal Investigator; Robert E. Burke, M.D.

Other Investigators: William Z. Rymer, M.D., Ph.D.
John V. Walsh, M.D.
Kenro Kanda, M.D., Ph.D.
Peter Tsairis, M.D.

Cooperating Unit: Department of Neurology, Cornell University Medical School, New York, N.Y.

Man Years:

Total:	4.4
Professional:	3.7
Other:	0.7

Project Description:

Objective: This project is designed to provide information in the following interrelated areas: (a) concerning the intrinsic properties of alpha motoneurons and the physiological, morphological and histochemical characteristics of the muscle fibers innervated by the investigated motoneurons; (b) concerning the patterns of usage of motor units of different types during a variety of movements in anesthetized and in alert, freely moving animals; (c) concerning the organization of synaptic input to alpha motoneurons from segmental reflex and descending systems; and (d) concerning the interaction of segmental and descending systems in the control of information flow in spinal segmental motor mechanisms.

Methods Employed: Anesthetized cats have been used in acute experiments involving intracellular microelectrode recording and stimulation techniques. Such methods permit evaluation of motoneuron electrophysiology and synaptic input together with recording of the mechanical response of the muscle fibers innervated by the same cell. The organization of synaptic input has been explored largely with the use of electrical stimulation of various peripheral nerves and supraspinal structures.

Major Findings:

A. Organization of synaptic input to motor units of defined type.

We have demonstrated that the population of motor units in the cat medial gastrocnemius muscle can be divided into categories on the basis of the mechanical properties of the muscle fibers belonging to the units. A precise relation exists between these categories and the types of muscle fibers present in this muscle, defined in terms of histochemical and morphological criteria. Ongoing investigation of the organization of synaptic input to the different classes of motor units from a variety of afferent sources has largely been devoted to examination of quantitative differences in input distribution to the various motor unit types. For example, if gastrocnemius units are ranked in order of decreasing amplitude of group Ia EPSPs, that half of the total population of units with larger than the mean Ia EPSPs are almost exclusively the slow twitch S and fast twitch FR units, both of which are quite resistant to mechanical fatigue. However, this half of the motor unit population produces only about 20% of the theoretical maximum tension output of which the whole muscle is capable. The motor units active in this range individually produce relatively small tensions and thus the summed motor output can be finely graded by recruitment of previously inactive units, as well as by modulation of their firing frequency. Over the higher 80% of the tension range contributed by units with Ia EPSPs smaller than the mean, the contribution of individual units to total tension becomes much larger, so that the system operates along a different slope with regard to cumulative tension per increment in number of units recruited. The units in this high tension range are for the most part type FF which have rather little resistance to fatigue because their muscle units depend on anaerobic glycolysis for energy.

Work has continued on the organization of other synaptic systems as they relate to motor unit type, taking the pool of motor units in the cat medial gastrocnemius muscle as a model for large limb muscles of heterogeneous motor unit composition. A variety of primary afferent and descending input systems are being investigated in order to define as fully as possible patterns of connectivity which must be involved in the control of various motor unit types during movements in intact animals. A particular effort has been made to assess the possible interaction occurring in segmental interneurons due to converging input from both primary afferent and descending systems. A previously described excitatory system of interneurons activated by skin afferents and projecting mainly to the fast twitch FF and FR motor units has been shown to be a discrete pathway not subject to inhibition from presumed reticulospinal pathways. This contrasts with the inhibitory pathway(s) from skin afferents to

gastrocnemius motor units, which are very effectively blocked by the reticulospinal action.

B. Effect of compensatory hypertrophy on the gastrocnemius motor unit population.

Compensatory hypertrophy of the medial gastrocnemius muscles has been produced by the denervation or surgical excision of all of the synergist ankle extensor muscles in a series of adult cats. After three to seven months' survival, the motor unit populations in each animal were surveyed using techniques previously developed in this laboratory. Analysis of the results has been complicated by the fact that individual animals had different degrees of hypertrophy because of differences in scarring and in some cases, actual contractures, which prevented full extension of the overloaded muscle. However, in animals which clearly had hypertrophic muscles, the motor unit populations showed no change in the percent composition with regard to the types of motor units present in normal animals, but all of the unit types showed a degree of increase in maximal tetanic tension production. Other physiological properties appeared to be essentially unaltered. Analysis of the same muscles by histochemical techniques is still underway by Dr. Peter Tsairis, Cornell University Medical School, who is collaborating on this project.

C. Usage patterns of single motor units in awake and freely moving animals.

For several years we have been attempting to develop a reliable method by which the discharge patterns of single motor units can be monitored over a period of days or weeks in freely moving animals. During the past year, some progress has been made utilizing an electrode system designed and fabricated by Mr. Martin Bak of LNLC. This project has proven to be extremely difficult from a technical viewpoint and has resulted in little usable data thus far. However, the potential for important advance is sufficiently great to merit continuation of the effort.

D. Analysis of the central effects of secondary muscle spindle afferents.

The central effects of secondary (group II) muscle spindle afferents on extensor alpha motoneurons has continued with exploration of the synaptic potentials recorded within motoneurons during muscle stretch and vibration, and during electrical stimulation of muscle nerves with and without conduction blockade of the larger group Ia primary spindle afferents. Thus far, the results continue to confirm the conclusion that the group II afferents may contribute polysynaptic excitation to extensor motoneurons in decerebrate animals.

E. Reviews.

During this fiscal year, Dr. Burke has completed a large scale review of spinal cord neuronal physiology for the Handbook of Physiology, co-authored by Dr. P. Rudomin of the National Politecnic Institute, Mexico City. A less extensive review of motor unit physiology and usage has been written in collaboration with Dr. V. R. Edgerton, UCLA.

Significance to Biomedical Research and the Program of the Institute: The problem of motor control in the intact animal involves consideration of the functioning of motor units as individual entities, active in asynchronous patterns. Much of the present work has been designed to further our understanding of the input-output relations of motor units, considered on the basis of single units rather than whole muscles or muscle nuclei. It can be expected that information regarding the organization of synaptic input to different groups of motor units within the same muscle will give a better picture of how the central nervous system may utilize these units during movements of various sorts. In addition, knowledge of the mechanical output characteristics of the same motor units permits inferences about the ultimate result of patterns of motor unit discharge in terms of muscle tension output. The motor unit has long been recognized as the ultimate target of integrative mechanisms in the motor system. The aim of this project is to contribute to a detailed understanding of the mechanisms, particularly those in and projecting to the spinal cord, which operate to control motor behavior.

Proposed Course of Project: We will continue with our analysis of the interaction of descending systems with segmental interneuronal arcs, concentrating particular attention on the rubrospinal and vestibulospinal interactions with the system of excitatory interneurons conveying input from skin afferents to fast twitch motor units. Material related to the histochemical effects of muscle hypertrophy will require extensive analysis over the next six to eight months. We will continue to explore techniques designed to permit recording single motor unit discharge in freely moving animals. Such techniques, if and when they become feasible, will greatly advance the study of motor systems in intact animals not only in our own project but in any similar application, potentially including studies in human subjects. The work on central effects of group II spindle afferents should be completed by the end of the present fiscal year.

Honors and Awards: Invited lecture (Dr. Burke) at an International Symposium on the Control of Posture and Locomotion, sponsored by the University of Alberta, Canada.

Publications:

Burke, R.E., Levine, D.N., Tsairis, P. and Zajac, F.E.: Physiological types and histochemical profiles in motor units of the cat gastrocnemius. J. Physiol. (Lond.) 234: 723-748, 1973.

Burke, R.E. and Tsairis, P.: Anatomy and innervation ratios in motor units of cat gastrocnemius. J. Physiol. (Lond.) 234: 749-765, 1973.

Burke, R.E., Rymer, W.Z. and Walsh, J.V., Jr.: Functional specialization in the motor unit population of cat medial gastrocnemius muscle. In Stein, R.B., Pearson, K.G., Smith, R.S. and Redford, J.B. (eds.): Control of Posture and Locomotion. New York, N.Y., Plenum Press, 1973, pp. 29-44.

Rymer, W.Z. and Walsh, J.V.: Effects of secondary muscle spindle afferent discharge on extensor motoneurones in the decerebrate cat. In Stein, R.B., Pearson, K.G., Smith, R.S. and Redford, J.B. (eds.): Control of Posture and Locomotion. New York, N.Y., Plenum Press, 1973, pp. 411-414.

Burke, R.E. and Tsairis, P.: The correlation of physiological properties with histochemical characteristics in single muscle units. Ann. N.Y. Acad. Sci. 228: 145-158, 1974.

Burke, R.E., Levine, D.N., Salcman, M. and Tsairis, P.: Motor units in cat soleus muscle: Physiological, histochemical and morphological characteristics. J. Physiol. (Lond.). In press.

Bergmans, J., Burke, R.E., Fedina, L. and Lundberg, A.: The effect of DOPA on the spinal cord. 8. Presynaptic and "remote" inhibition of transmission from Ia afferents to alpha motoneurones. Acta. Physiol. Scand. In press.

Rudomin, P., Nunez, R., Madrid, J. and Burke, R.E.: Primary afferent hyperpolarization and presynaptic facilitation of Ia afferent fibers induced by low threshold cutaneous afferents. J. Neurophysiol. In press.

Serial No. NDS(I)-69 LNLC/OC 1687
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Techniques for Making Connections with the Nervous System

Previous Serial Number: Same

Principal Investigator: F. T. Hambrecht, M.D.

Other Investigators: Martin Bak
Karl Frank, Ph.D.
*Seth Goldstein, Ph.D.
George Laird
Gerald Loeb, M.D.
William Marks, Ph.D.
W. Zev Rymer, Ph.D.
**Michael Salcman, M.D.
Edward Schmidt, Ph.D.

Cooperating Unit: *Division of Research Services - Biomedical Engineering and Instrumentation Branch
Serial No. DRS-BEIB-12

**Columbia-Presbyterian Medical Center
New York, New York

Man Years:

Total:	4.5
Professional:	2.3
Other:	2.2

Project Description:

Objectives: Successful applications of neural control require the development of techniques for making connections to the nervous system. These include both acute and chronic techniques for recording, stimulating and inhibiting individual nerve cells or small groups of cells of similar function.

Methods Employed:

A. Materials suited for biological implantation. Iridium has been added to the group of metals which are beirg evaluated in animals for the stability of their physical properties and

their biocompatibility. Evaluation of cyanoacrylate adhesives, gold, tantalum, epoxy, Parylene C, teflon and certain medical grade silastics has continued.

B. Designs for a chronic recording intracortical microelectrode. Development of the "map pin" electrode has continued. In particular, the tip insulation is now removed by exposing it to a high A.C. voltage arc across a 600 micron air gap. After implantation, the electrode position can be changed slightly if desired by temporarily opening the acrylic chamber to atmospheric pressure. This permits pulsatile movement of the brain and slight movement of the electrode relative to the brain tissue. Three monkeys and six cats underwent implantation of sets of these electrodes during the past year.

C. Microelectrode introducer capable of tracking the moving brain. Development of a microelectrode introducer for use in obtaining single unit recordings from the pulsating brain during neurosurgical procedures has continued. Preliminary testing has been conducted in monkeys with large craniotomies. The device is now ready for testing in humans.

D. Capacitor stimulating electrode. Chronic testing of capacitor electrodes has been initiated by contractors in the Neural Prosthesis Program. Members of the Laboratory have assisted these contractors, as well as other investigators in other laboratories at NIH, in setting up experiments which take advantage of their safe, stimulating properties. A commercial supplier has been established to supply the demand for these electrodes.

E. Groove electrode arrays for chronic recording from nerve fibers. The diameter of the grooves has been increased to 300 microns in an attempt to increase the survival of nerve fibers. Both cat dorsal root filaments and rabbit muscle nerve fibers have been tested in the grooves. For chronic testing, a silastic button percutaneous connector has been used.

F. Multipolar electrode for single motor unit recording in muscle. This technique relies on the observation that each motor unit consists of fibers distributed uniquely within a large portion of the muscle and that at any one point in the muscle, the time of occurrence and the frequency distribution of a signal recorded at a microelectrode is dependent on the distance of the electrode from the end plate and the distance of the electrode from the muscle fiber surface. A special array of six microelectrodes consisting of gold leads with short, welded-on barbs of 12 micron iridium wire is inserted by pulling it through the muscle and tugging slightly backward to lodge the barbs in place. The signals from up to four of the barb electrodes are differentiated,

variably delayed, and mixed with weighted coefficients until a single unit is readily identifiable above the background noise. The signals from the electrodes may be recombined in various ways to identify several different units from the same data channels, and the mixing parameters and shape of the separated unit recorded to allow future recognition of the unit when individually stimulated.

G. Chronic single-unit recordings from dorsal root ganglia. Methods: A variation of the successful free-floating "map pin" electrode for chronic cerebral cortex recording developed in this laboratory is proposed. This electrode will be specially designed to overcome the very tough connective tissue sheathing of the dorsal root ganglia and the limited space available for multiple probes both on the ganglia and in their narrow bony canal. Short pointed electrodes with Parylene insulation will be welded to ultraflexible gold leads which can be left coiled in the epidural fat around the ganglia and brought out through a small laminotomy to a back pack connector.

H. Forced aliquot release technique. Development of an implantable, remotely controlled fixed unit dose injector which will release a 5-50 microliter aliquot of drug from a reservoir into a distant tissue space has begun. The reservoir with an injection port is contained inside a concentric rigid enclosure holding a volatile, inert liquid which is in equilibrium with its gas phase at a constant pressure in excess of atmospheric pressure. The drug solution in the reservoir is led off through a narrow gauge capillary to give a potential constant flow rate and then to an electrically controlled valve consisting of a solenoid driven rod which crimps a soft silicone rubber tube. The negative feedback control information is generated near the delivery tip of the teflon catheter which leads from the main package to the site of injection. There, three platinum wires end in loops along the inside wall of the tube. Two closely spaced loops about 2 cm from the tip are used to generate a bubble by electrolysis of the saline solution containing the drug. A third loop near the tip detects the impedance change in the column of fluid caused by this bubble and gates the constant flow rate pump on until the bubble reaches the tip and the impedance falls. The bubble remains lodged at the tip acting as a diffusion barrier to prevent leakage of drug from the column of fluid in the tube until the next dose is desired.

Major Findings:

The long-term stability of Parylene C during implantation in the brain is in doubt. The impedance of electrodes insulated with this material has fallen by more than an order of magnitude during a seven month implant period.

Single unit activity has been recorded from eleven of twelve "map pin" electrodes for over six months. Although the activity of one identified neuron was followed for more than three days, there is evidence that motion exists between the electrode and the cells even after months of implantation.

Using the microelectrode introducer to record from exposed, pulsating cortex, single units have been held for periods exceeding 30 minutes. During one of these periods, the device tracked cortical pulsations up to 1 mm.

The groove electrode array has proven to be electrically stable in situ for at least one week. However, nerve filaments in the grooves fail to conduct impulses after about 24 hours in the cat and after three days in the rabbit. This is felt to be due to a decrease in blood circulation in the region of the implant.

Identification of motor units in muscle which were not recognizable on individual data channels was demonstrated using the multipolar electrode and analog processing.

Significance to Biomedical Research and the Program of the Institute: The successful development of techniques for recording signals from the nervous system and means of selectively controlling the activity of small populations of neurons is mandatory for the success of the Laboratory's objectives. Such developments are also of value to the neurophysiological research of other laboratories, including those working on prostheses for the neurologically handicapped.

Proposed Course of Project: The microelectrode introducer is now ready for clinical trials in epileptic patients. It will be used in the NIH Clinical Center to localize foci of seizure activity for surgical resection. The work on the capacitor electrode for cortical surface stimulation is essentially completed and is being used in commercially available stimulation devices. The remaining projects discussed need further development and work will continue on them. Further cooperation with contractors in the Neural Prosthesis Program is anticipated.

Honors and Awards: None

Publications: Guyton, D.L. and Hambrecht, F.T.: Theory and design of capacitor electrodes for chronic stimulation. Med. Biol. Eng., 1974. In press.

Serial No. NDS(I)-69 LNLC/OC 1688
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cortical Mechanisms of Voluntary Motor Control

Previous Serial Number: Same

Principal Investigator: Edward M. Schmidt, Ph.D.

Other Investigators: Martin Bak
George Dold
Karl Frank, Ph.D.
Joan McIntosh

Cooperating Units: None

Man Years:

Total:	2.6
Professional:	1.0
Other:	1.6

Project Description:

Objectives: The major goal of this project is to study the feasibility of using cortical neurons for the control of external devices such as a prosthesis. This involves the study of relationships between the firing patterns of cortical neurons and the measurable parameters associated with simple movements. Our aim is to determine whether it is possible to extract enough information from the spike trains of small sets of cortical neurons to be able to predict the time course and amplitude of various motor response measurements. This requires a careful study of the types of cells to be found in the motor cortex: their frequency of occurrence, location and specific function. At the same time, we are anxious to determine how much control an individual has over the firing patterns of these cortical cells. If an individual can influence the firing rate of specific neurons, the chances for building a centrally controlled prosthesis are markedly improved.

Methods Employed: Monkeys were initially trained to move a handle to obtain a liquid reward. After implantation of up to a dozen "map pin" chronic microelectrodes in the motor cortex, the animals were allowed to make free arm movements and were given liquid rewards dependent on the firing patterns of individual cells.

Major Findings:

1. Monkeys were required to modify the firing rate of single cortical cells to obtain a juice reward. During approximately one half of the recording sessions, the animal could significantly alter the firing rate of the cell. The cells whose rate could easily be modified were usually associated with a specific arm movement. Only rarely could firing rates be modified if they were not associated with limb movement.

2. Recordings from the same cortical cell for up to a week have been obtained with the chronic "map pin" electrodes. However, relative movement occurs between the cell and electrode that limits the time the same cell can be observed. This relative movement was still occurring seven months after electrode implantation. The source of this motion as yet is unknown and remains a topic for future investigations.

3. With long-term chronic microelectrode implantation, we are finding that our insulating material, Parylene C, which was considered stable in vivo, has shown considerable degradation. When the current animal is sacrificed, the electrodes and brain tissue will be examined to determine what is happening. Either different methods of removing the Parylene at the electrode tip are required, or a different insulating material is required.

Significance to Biomedical Research and the Program of the Institute: Through our studies we are obtaining a better understanding of the function of the motor cortex and the types of control signals we can obtain. We are quite optimistic that long-term chronic recordings can be obtained from the motor cortex and these signals used to control the stimulation of paralyzed muscles or control a prosthetic device.

Proposed Course of Project: Work will continue in the area of obtaining long-term chronic recordings from cells of the motor cortex. The degree to which the firing patterns of cells can be controlled will be examined in refined tasks to determine if proportional control of an external system is possible with single cortical cells. The degree to which several cells can simultaneously control an external device will also be investigated.

Further studies will be conducted on the cause of what appears to be movement between the cell and electrode tip. The duration that recordings may be obtained from a single cell will greatly influence the type data processing that will be required to use the signals for control of a prosthetic device.

Insulating material studies will also continue until we find a substance that remains stable in the body and does not cause adverse tissue reactions.

Honors and Awards: None

Publications:

Schmidt, E.M., Bak, M.J. and McIntosh, J.D.: Cortical cell conditioning with permanently implanted microelectrodes. Fed. Proc. 33: 1062, 1974.

Schmidt, E.M., Jost, R.G. and Davis, K.K.: Plasticity of cortical cell firing patterns after load changes. Brain Res. 73: 540-544, 1974.

Serial No. NDS(I)-69 LNLC/OC 2015
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural Prosthesis Program

Previous Serial Number: Same

Project Officers: Frederick T. Hambrecht, M.D.
Karl Frank, Ph.D.

Other Investigators: Herbert C. Lansdell, Ph.D.

Cooperating Unit: C&FR - NINDS

Man Years:

Total:	2.9
Professional:	1.9
Other:	1.0

Project Description:

Objectives: An attempt is being made to combine recent advances in basic neurosciences, natural sciences and microelectronics for the development of prostheses for the neurologically handicapped. In particular, the feasibility of a sensory prosthesis for the blind utilizing direct electrical stimulation of the visual cortex, a bladder prosthesis for patients with neurogenic bladders utilizing electrical stimulation of the sacral spinal cord, functional neuromuscular stimulation with intramuscular electrodes in patients with upper motor nueron lesions and evaluation of the effects of chronic cerebellar stimulation for relief of medically intractable epilepsy are being investigated.

Methods Employed: The feasibility studies are being carried out primarily through contracts with eight institutions. These contracts are budgeted and administrated mainly through C&FR. Project Officers and intramural collaboration are within LNLC-IR.

Functional Neuromuscular Stimulation: Case Western Reserve University has implanted intramuscular electrodes in paralyzed patients and is studying the degree of proportional control that can be achieved. Sequential stimulation through multiple electrodes is utilized to provide smooth muscle contractions and to reduce fatigue. They are also studying the effects of electrical stimulation on the physiological and histological properties of muscles in both normal and spinal animals.

Bladder Evacuation: Both Duke University and the University of California at San Francisco are stimulating the spinal cords of both normal and spinal animals to determine if cell populations exist in the cord which can be selectively stimulated to cause bladder detrusor contraction with simultaneous sphincter relaxation.

Sensory Prosthesis: The University of Rochester, using sighted and blinded rhesus monkeys, is studying methods of optimizing information input to the visual cortex, survival of electrical excitability during long-term stimulation, and the effects of blindness on these factors.

The Massachusetts General Hospital is investigating the relationships between stimulus parameters and afterdischarges following electrical stimulation of the visual cortex in animals. They are also studying the effects of changes in electrode parameters and electrode size by stimulating visual cortex of human subjects who require occipital craniotomies.

Cerebellar Studies: A request for proposals has been released for a new contract to study the effects of cerebellar stimulation for prevention or termination of seizures in primate models of epilepsy.

Basis Studies: Environmental Impact Center is determining the electrochemical reactions at the electrode-electrolyte junction during the passage of current and methods of identifying and minimizing possible toxic byproducts.

The Huntington Institute of Applied Medical Research is concentrating on developing methods of confining current spread during electrical stimulation. They are also continuing their series of chronically stimulated animals with subsequent histopathological examination of surrounding tissue.

The University of Florida is performing long-term in vitro tests of potential electrode materials using scanning electron microscopy and atomic absorption spectrophotometry to evaluate corrosion of these electrodes during electrical current passage. Promising materials are used as chronic stimulating electrodes in animals with subsequent histopathological study of surrounding tissues.

The Project Officers have coordinated some of their research activities at NIH with those of the contractors and have organized a workshop, now held annually on neuroprostheses.

Major Findings: No practical level of electrical stimulation of the visual cortex in macaques has been found which does not produce at least a temporary increase in threshold for detection by the animal. At threshold currents for producing an afterdischarge with cortical surface stimulation in the cat, the afterdischarge involves a region of the brain around the stimulation electrode with a radius of approximately 2 mm. With suprathreshold stimulation, this radius can increase to at least 4 mm.

Thin, unreinforced silastic electrode arrays have developed a curling distortion resulting in cortical compression after implantation on the surface of cat cortex. Histologically, neuronal displacement occurs with little or no neuronal loss.

During multiple, symmetrical, biphasic, constant current pulsing passed into simulated cerebrospinal fluid, the potential of a platinum electrode tends to oscillate around the hydrogen potential. Electrode corrosion as indicated by increases in real surface area has also been noted.

Platinum-10% iridium and gold as materials for stimulating electrodes are unacceptable due to severe pitting and crevice corrosion. Titanium oxide and tantalum pentoxide have shown excellent corrosion resistance, most likely due to their passive oxide surfaces.

Active bladder contraction is easily achieved during electrical stimulation of the sacral spinal cord in dogs. However, no region of the spinal cord has been found which when stimulated results in relaxation of the striated urethral sphincter.

Continuous electrical stimulation for periods as short as 12 days is sufficient to increase the fatigue resistance of cat tibialis anterior.

A forearm orthosis using sequential electrical stimulation through three electrodes placed in the flexor digitorum superficialis and profundus of a C5 quadriplegic patient was evaluated. Utilizing a proportional output shoulder position transducer to control stimulation, the patient could independently grasp and use items such as pencils, cups, combs and eating utensils.

Significance to Biomedical Research and the Program of the Institute: As these feasibility studies continue, the possibility of developing truly useful neural prostheses for the neurologically handicapped becomes more promising. The results of these studies are already proving useful to current neurosurgical techniques involving electrical stimulation and manufacturers of equipment for

such stimulation have utilized results from these studies for the design of their equipment. Studies on the effects of cerebellar stimulation for relief of intractable epilepsy will be useful both for the Epilepsy Program of the Institute and for neurosurgeons who are attempting to use this technique clinically.

Proposed Course of Project: Further studies of the type indicated above are required, as well as investigation of the feasibility of new types of neural prostheses as basic research suggests possible modes of operation. Fundamental to all the neural prostheses presently being investigated is the further development of safe, effective means of selectively exciting or inhibiting small populations of neurons.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNLC/OC 2078
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Input-Output Pathways of Dorsal Root Ganglion Cells

Previous Serial Number: None

Principal Investigator: Gerald E. Loeb, M.D.

Other Investigators: None

Cooperating Unit: None

Man Years:

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description:

Objectives: Evidence from a number of investigators is mounting to indicate that the classical interpretation of dorsal root ganglion cells with a peripheral branch coursing distally via a single spinal nerve and a central branch coursing proximally via associated dorsal root is incomplete. There are known to be ganglion cells scattered in the ventral roots; spinofugal fibres carrying primary afferent signals have been identified in dorsal roots; and a large proportion of unmyelinated fibres in the ventral roots have been found to have cell bodies in the dorsal root ganglia. This project is designed to identify and quantify these and possibly other unusual pathways of processes from somata in the dorsal root ganglia.

Methods Employed: Ganglion cells are identified by extracellular single unit recordings from their somata by specially designed dual glass and platinum-iridium microelectrodes. Their receptor type and conduction velocity in sciatic nerve are identified and processes are searched for by stimulation of spinal nerves and dorsal and ventral roots at the same and adjacent higher and lower levels. At present, all ganglion cell recordings are being made from the L7 ganglion of the cat.

Major Findings: Electrodes have been devised which have successfully penetrated the dense connective tissue of the ganglion

without harmful dissection. Stable extracellular single unit potentials of 50-500 microvolts have been recorded from ganglion cell bodies while differentially removing the large evoked potentials produced by synchronous activation of densely packed cell bodies on electrical stimulation. About 20 cells with peripheral conduction velocities of 20 to 80 m/sec. have been identified with various receptor types, and no unusual processes have yet been identified. Work is just beginning on stimulating and recording from cells whose fibres conduct in the 0.5 to 2 m/sec. range, where most of the unusual connections are believed to arise.

Significance to Biomedical Research and the Program of the Institute: The ganglion cells whose pathways are non-classical are suspected of being in some way unique in their function and may subserve neurophysiologic processes not yet identified. Among the suggestions in the literature are that they represent an information processing system utilizing ephaptic modulation of safety factors to integrate and store information; that they are not actually primary afferents but rather interneurons communicating between the dorsal and ventral parts of the cord; that they are a special class of small fibre afferents which make unusual connections on the corticospinal or motoneuron population in the ventral horn. In addition, a quantitative assessment of these pathways is of particular interest to investigators utilizing the generally accepted technique of dorsal rhizotomy to achieve complete deafferentation.

Proposed Course of Project: A series of the acute experiments described will be done to quantitate any unusual pathways identified in terms of the percent of all ganglion cells sampled and distribution of conduction velocity and receptor type. Parallel investigations utilizing histochemical techniques such as radioactive amino acids and peroxidase uptake are planned to confirm any pathways which seem significant.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNLC/OC 2079
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Models of Neural Interactions

Previous Serial Number: None

Principal Investigator: William B. Marks, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: The primary objective of this project is the generation of a model of a part of the nervous system capable of detecting special features of the information it receives. One commonly observes in the nervous system a group of nerve cell bodies, each receiving a different mixture of excitatory and inhibitory connections from a shared group of afferent nerve fibers. It appears useful to hypothesize that each neuron of such a group is indicating by its firing rate the amount of some independently varying property, or "feature", of the patterns of activity in the afferent fibers. It is proposed that the connections for accomplishing this are not determined entirely genetically, but that during development the synaptic strengths are influenced by the afferent activity. If the activity of these neurons is correlated then they are not detecting independently occurring features of the afferent patterns. Therefore, the synaptic strengths should change in a direction which tends to reduce the correlations in the activities of the cells.

A justification for seeking such a model is the observation that the cells of the mammalian visual cortex appear to detect independently varying features of the patterns of activity coming from the retina. A further goal of this project is to present a series of visual patterns to the modeled system and to compare the features it discovers to the naturally occurring

feature detectors of the visual cortex. Features of the patterns of activity from muscle and joint afferents during limb movements might also be studied using such a model for comparison with naturally occurring afferent connections on the cells of the spinal cord.

Methods Employed:

Matrix algebra and laplace transform methods are used, implemented on the NIH PDP-10.

Major Findings:

A. The field of Factor Analysis is also concerned with the problem of finding significant variables underlying a number of correlated variables ("data"). It also solves the problem by forming linear combinations ("mixing") the data in such a way that the mixtures are uncorrelated. However, the measure of correlation used in Factor Analysis, covariance, allows many solutions. There are many ways to mix the data so that the mixtures have zero covariance. Factor Analysis eliminates extra solutions by making assumptions about the result. I have found a way which does not require any such assumptions, but which utilizes only the original concept of independence. It is required not only that the mixtures have zero covariance, which is the expectation of the product of a pair of mixtures, but also that the product of any group of three of the mixtures has a zero expectation. This leads to the discovery that the proper mixtures can be computed from the expectations of products of the data taken three at a time.

This is relevant, since the application of Factor Analysis alone to the construction of a model visual system would probably lead to both observed and unobserved receptive fields. For example, Factor Analysis would probably predict cells with crossed "simple" receptive fields, one excitatory, one inhibitory, unless additional artificial constraints were imposed.

B. A nerve net was constructed in which the synaptic strengths were modified if the activity of the cells were correlated. This successfully led to connections for which the activities were uncorrelated.

C. The nerve net of B probably is satisfied by many receptive fields which do not occur in the visual system, since it utilizes only covariance as a measure of correlation. Since the computation technique of A is too complicated to be embodied in a simple nerve cell net, a simpler criterion for correlation was sought. It was found that if not only the covariances of

the activities of the cells were zeroed, but also the covariances between each cell and the square of the activity of every other cell, the extra solutions would be eliminated.

D. A second neuronal net was constructed for which inputs become more potent onto cells whose activity they correlated and in which cells with correlated activities increased their mutual inhibition. The cells came to detect the features of a number of sets of patterns presented to them, including the bars required to construct the presented patterns: the digits ɔ-9.

Significance to Biomedical Research and the Program of the Institute: These models provide a well-defined hypothesis which makes a clear prediction about the strengths of all the connections in the nervous system and about the "learning" that could occur in each part of the nervous system. It is that every cell or functional grouping of cells tends to lock into a "feature" to the input pattern that is available to it. This may lead to predictions about e.g. the patterns detected by cells of the ventral spinocerebellar tract: That these cells are detecting "features" of the movement of the limb as a whole.

Proposed Course of Project: The results to date will be submitted as four papers for publication. The results in B and C of "Major Findings" will be utilized to create a set of growth rules for synapses. Visual patterns will be presented to the model, and the resulting receptive fields will be compared with natural ones. A similar problem will be initiated utilizing limb movements as sources of correlated neuronal activity. A review will be attempted in which the visual system and other systems, including ones of the spinal cord, will be discussed in terms of the hypothesis that neurons detect features.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LNLC/OC 2080
1. Laboratory of Neural Control
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neuron Activity during Locomotion

Previous Serial Number: None

Principal Investigator: William B. Marks, Ph.D.

Other Investigators: Joaquin-Andres Hoffer
George Laird
George Dold
William Z. Rymer, M.D., Ph.D.

Cooperating Unit: Johns Hopkins University, Biophysics Department, Baltimore, Md.

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Objectives: This project has several related objectives: a. to obtain information about the roles of spinal neurons in controlling locomotion, b. to develop preparations which utilize our electrodes for monitoring and stimulating neurons of the unrestrained animal, and c. to develop techniques and concepts for dealing with patterns of simultaneous neuron activity. We are particularly interested in the role of muscle afferent fibers in controlling the movement of the limb, and in the degree to which motoneuron activity reflects the "expected" movement of the limb.

Methods Employed:

A. Electrodes. At present we are most hopeful about our "groove electrodes" for capturing bundles of dorsal and ventral root fibers and peripheral nerves. Groove electrodes are described in the Project Report "Techniques for making connections with the nervous system," No. NDS(I)-69 LNLC/OC 1687. These electrodes appear to be insensitive to movement and are usable in multichannel arrays. Extracellular metal electrodes for dorsal root ganglion cells, developed by Dr. Loeb, may also be available. It may be possible to record from cells of the

spinal cord using flexible wire electrodes similar to those of Martin Bak and co-workers of the Laboratory.

B. Spike waveform recognition. The activity from the groove arrays, and perhaps from the extracellular electrodes, will contain waveforms from several neurons. These will be distinguished automatically, either by their basic waveform content (Marks, 1965; Glaser and Marks, 1967), or by their amplitudes at chosen times (Schmidt, 1972).

C. Locomotion: Recording and analysis. The animals will run or walk on a treadmill before a high-speed video recording system. Joint angles will be computed from the position of tags attached to the animal, which will be automatically tracked by electronics within the video system, or by a separate solid state photoposition monitor. Footfall times will also be measured.

D. Transmission and recording. The multichannel neuronal signals will be relayed from the animal by a portable 10-channel transmitter made for us by the Jet Propulsion Laboratory. The multiunit data, the separated single unit data, and the locomotor movement data will be logged on a 14-channel tape recorder.

E. Computations. We will attempt to partition the unit activities and the limb movement variables into correlated groups using the PDP 12. For this purpose, factor analysis techniques may be required, perhaps as modified by Marks (see Project "Models of neural interactions"), using the PDP-10.

F. Anatomy. The type and termination of the recorded units will be verified in a terminal acute experiment by dissection, conduction velocity, vibration, and other techniques.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: A practical technique for chronic contact with small bundles of spinal root fibers should be useful in restoring movement to paraplegics. Signals derived from some remaining voluntary channels could be applied to the ventral roots (calculations show that this is the most efficient electrode configuration for stimulation) and the muscle and joint afferents of the dorsal roots could provide essential feedback on the actual results of stimulation. Tactile feedback would also be valuable.

The information gained from signals generated by neurons controlling the movements of free animals should be invaluable in deciphering the mechanism of this control, because other

methods are compromised in an unknown manner by the effects of anesthesia, immobilization, or preparative lesions.

Proposed Course of Project: As described under "Methods Employed."

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Stroke

M.G.F. Fuortes, M.D., Chief

Seventeen scientists worked in the LNP during the past year. Five of these joined the Laboratory as visiting fellows or guest worker continuing a program of exchange and collaboration with other Departments which had been initiated in previous years.

Research activities included investigations on mechanoreceptors; visual pigments; retinal interactions; retinal degenerations; membrane properties of neurons; and development of muscle cells.

The studies on mechanical transduction were performed on the hair cells in the vestibular organ of the mollusc Hermissenda. This work provided the first satisfactory records ever obtained from receptors of this type. Kinetic analysis suggests that the responses originate in the cilia and arise, not as a direct consequence of the deformation of the membrane but rather as a result of a succession of temperature-sensitive transformations. The nature of these processes is still unknown.

Spectrophotometric techniques for investigation of visual pigments have been further developed. Research performed with polarized light leads to the conclusion that the chromatophore in vertebrate photoreceptors is precisely oriented relative to the visual axis, with the chains of conjugated double bonds positioned in the appropriate way for maximal absorption of the light.

The retinal studies have yielded interesting if unexpected results. It has been seen that, under certain conditions, cones develop depolarizing, rather than hyperpolarizing responses to light. This happens whenever a cone does not absorb light but is surrounded by cones which do. In these conditions the cone in question receives depolarizing impingement from horizontal cells without itself developing a direct hyperpolarizing response. Thus green- and blue-absorbing cones are depolarized by red lights. These observations have led to a new interpretation of the color-dependent responses of the chromatic horizontal cells.

Comparison of the responses of rods and cones in the salamander shows that light has opposite effects on the current-voltage curves of these two cells. The results suggest that light decreases membrane conductance in the cones but increases it in the rods. It is not known if the observed effects originate in the outer segment or involve other parts of the cell membrane.

The results on the retina have aroused considerable interest. Members of the LNP were invited to organize a Symposium on their results at the 57th Meeting of the Federation of American Societies for Experimental Biology and participated as invited lecturers to conferences in the U.S. and abroad.

Retinal degeneration resembling conditions found in humans has recently been discovered in colonies of albino rats. The process causes total loss of photoreceptors and has an incidence of 73 percent in certain colonies. It is hoped that investigation of this degeneration may help to understand the causes of some retinal distrophies in man.

Work on molluscan neurons demonstrates that the rhythmical activity of the "bursting" cells of the snail is caused by voltage- and time-dependent characteristics of the ionic permeabilities of the membrane. At a certain voltage the membrane is specifically permeable to sodium and, therefore, tends to depolarize; depolarization causes decrease of sodium and increase of potassium permeability to sodium. By these mechanisms the cell develops regular oscillations with bursts of impulses during the depolarizing cycles.

The development of membrane properties of chick muscle cells has been studied in tissue cultures. It has been demonstrated that these cells produce three types of depolarizing activity: a rapid spike due to entry of sodium; a slower spike due to calcium influx; and a very slow wave caused by outflux of chloride ions. There are reasons to suspect that the slow chloride transient, readily revealed in tissue cultures, may be a more widespread type of activity than is usually recognized.

The results of the studies outlined above have been described in twelve articles published during the year.

Serial No.:NDS(I)-65 LNP/SP-1239

1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Photoreceptors in the Limulus Eyes

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: The objective of this research is to gain insight into the means by which light is transduced into an electrical current in photoreceptors. The intracellularly recorded potentials and currents of the photoreceptor cells of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods Employed: Responses are recorded with intracellular microelectrodes and analyzed under voltage clamp. Using this technique, the role of visual pigments, of photoreceptors cell membrane and of various ions in photoreception can be studied.

Major Findings: The investigations have been concerned with an analysis of the membrane properties of Limulus photoreceptors in the dark, in steady light, after their treatment with metabolic inhibitors (strophanthidin), and after alteration of the extracellular fluids divalent cations. Using the voltage clamp technique, Limulus photoreceptor membranes have been found to have an N-shaped steady-state current-voltage (I.V.) curve. The properties of this I-V curve change with the state of light or dark adaptation of the photoreceptor and hence with its photic excitability. Specifically, the membrane I-V curve has the largest region of negative slope when the cell is fully dark adapted; light adaptation reduces or eliminates the region of negative slope; and the negative slope returns with subsequent

dark adaptation. The N-shape I-V curve also embues the photoreceptors with electrically excitable properties, viz, an all-or-none "spike", which can be evoked in the dark-adapted but not in the light-adapted cell.

It has been known that calcium ions regulate photoreceptors' excitability to light, viz, low Ca^{++} enhances and high Ca^{++} reduces excitability. Alteration of calcium on the extracellular fluid also changes the photoreceptor's steady state I-V curve.

During the course of other and unrelated experiments (see project entitled "Electrophysiology of Simple Cellular Systems," NDS(I)-73 LNP/SP-2019), the bursting pacemaker activity of molluscan neurons was found to correlate with a steady-state N-shaped I-V curve. Moreover, the activity and the I-V curves were significantly altered by manipulation of divalent cations in the extracellular fluid. Specifically, lowered Ca^{++} enhanced the bursting activity and increased the negative slope region of the I-V curve; increased Ca^{++} had the opposite result. Moreover, Sr^{++} and Ba^{++} had effects similar to reduced Ca^{++}, while Co^{++} acted like increased Ca^{+}.

The parallels between the two systems, Limulus photoreceptors and molluscan bursting neurons, was sufficiently suggestive that the effects of Sr^{++}, Bu^{++} and Co^{++} on the Limulus late receptor potential were examined. Both Sr^{++} and Ba^{++} increased while Co^{++} reduced the amplitude of the steady state receptor potential.

Significance to Bio-medical Research and the Program of the Institute: The results add further confirmation that those models of photoreceptor function based either on linear and time-independent processes or on monovalent ionic conductance changes involving only one ionic species are inadequate to account for the properties of Limulus ventral eye photoreceptors and for the action of light, of the cardiac glycosides and of divalent cations on these cells. Moreover, the similarity of effects of divalent cations on photoreceptors' excitability and on other neurons' activity suggests that similar membrane mechanisms may be involved in achieving physiologically quite distinct function. If any such generality of mechanisms could be established, it would be of considerable scientific interest.

Proposed Course of the Project: It is proposed to continue the study of membrane properties of Limulus photoreceptors by the voltage-clamp techniques. These studies will also include further investigations of the effects of altered ionic environment and of other metabolic inhibitors on the excitability and the I-V curves of photoreceptors. Hopefully such experiments will provide information on which ionic species carry and

regulate the current through the time-independent and the various time-dependent conductances.

As pointed out in the Major Findings section, a number of the membrane properties which have been the object of study in this project appear to be similar to those found in experiments in another project. It would appear justified, therefore, to terminate this more limited project and include future research work on Limulus photoreceptors in the more general project, the Electrophysiology of Simple Cellular Systems. Such an arrangement would not only allow the use of the Limulus photoreceptor when its properties make it the optimum preparation, but also contribute to our understanding of the degree to which membrane mechanism found in other excitable cells play a role in photoreception.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/SP-1690
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Rapid Scanning Microspectrophotometry in Visual Cells

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: Franco Malerba, Ph.D.[3]
Theodore Colburn, Ph.D.[2]
Ferenc Hárosi, Ph.D.
George C. Murray, Ph.D.[1]

Cooperating Units: [1]Office of Associate Director, C&FR/NINDS
[2]Section on Technical Development, NIMH-NINDS
[3]C.N.R., Laboratorio Di Cibernetica E Biofisica, Camogli, Italy

Man Years:

Total:	1.6
Professional:	1.5
Other:	0.1

Project Description:

Objectives: The objective of this research is the development of rapid scanning microspectrophotometric techniques for investigation of the chemical kinetics within single excitable cells. Although these techniques are being developed to study the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will also be considered.

Methods Employed: The microspectrophotometer samples transmittance at spectral wave bands between 400 and 600 nm, either sequentially or in random order, at rates of 33 microseconds per sample point. The system uses a rapid scan monochromator in which the entrance slit has been replaced by the image of a cathode ray tube face.

Major Findings: During the past year the instrument was

tested out by using objects of known spectral composition in order to define the operating characteristics of the system. A number of the instruments' components were found inadequate and had to be re-designed and constructed. The main remaining technical problem is the control of the stability of the CRT light intensity with time. Currently efforts are in progress to overcome this technical problem. At present, the system is capable of measuring the absorption at a single wavelength within 0.6 milliseconds, has a wavelength resolution of 10 nanometers, and can detect absorption differences of 1-5%. Thus, the instrument can scan the spectrum from 400-600 nm in 10 nm increments in 120 msec. with adequate resolution for many biological experiments.

In addition to work on the instrument itself, computer programs have been written, tested out and used successfully to A/D convert and analyze the data on the PDP-12 computer.

A number of biological experiments have been performed on the isolated frog retina. The steady state spectra of rhodopsin and some of its breakdown products measured rapidly with this machine at physiological temperatures agree well with the spectra measured on slower instruments at lower temperatures. In addition, data have been obtained on the kinetics of some of the visual pigments; however, the accuracy of these data is questionable due to the aforementioned temperature instability of the light intensity.

Significance to Bio-medical Research and the Program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular systems functioning within living cells, for which an increasing need has developed.

Proposed Course of the Project: High priority is to be given to the development of means of improving the control of the CRT light intensity with time. Such control is essential if the instrument is to be fully useful in measuring the kinetics of visual pigments. Once adequate control is obtained, the proposed study of the kinetics of visual pigments will proceed.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-73 LNP/SP-2019
1. Laboratory of Neurophysiology
2. Section on Sensory Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrophysiology of Simple Cellular Systems

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr., M.D.

Other Investigators: Jun Fukuda, M.D.
Phillip Nelson, M.D., Ph.D.[1]
Harold Gainer, Ph.D.[1]
Jeffery Barker, M.D.[1]
Gerald Fischbach, M.D.[2]

Cooperating Units: [1]Behavioral Biology Branch, NICHD
[2]Department of Pharmacology, Harvard Medical School

Man Years:

Total:	2.8
Professional:	2.7
Other:	0.1

Project Description:

Objective: The objective of this research is to gain insight into the membrane mechanisms which are responsible for the activity of single cells.

Methods Employed: The electrical properties of single cells are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. The technique of voltage-clamping the membrane potential of single cells is the main method employed in these experiments. This technique requires the penetration of single cells with two micro-electrodes, one for monitoring the membrane potential, the other for supplying the current necessary to clamp the membrane potential at the desired level. Using this technique the role of the various membrane ionic conductances responsible for the electrical responses can be studied.

Because of the necessity of taking many measurements from each experiment, the data is often recorded on magnetic tape and analyzed with a PDP-12 computer.

Major Findings: This project is the outgrowth of two pilot studies begun two years ago. The first study involved an investigation of the electrical characteristics of chick muscle cells grown in tissue culture. The second was an analysis of the electrical properties of an identified, neurosecretory pacemaker cell in the ganglion of a snail.

A number of electrical properties of the chick muscle cells have been identified and traced during the course of development of these cells from their immature, myoblastic stage to an apparently "mature" stage. One particularly interesting electrical response in these cells is a slow "spike" associated with a slow contraction of the muscle. This slow spike appears quite early in development and changes with maturation. The results of electrophysiological investigations on this slow spike indicate that it is generated by a voltage and time dependent change in the muscle membrane's chloride permeability. Thus, the chick muscle has three types of spikes: 1) a conventional, fast sodium spike, 2) a slower calcium spike and 3) a very slow chloride spike.

The aim of the study of the snail ganglion cell was to see if the membrane mechanism underlying the bursting, pacemaker activity of the cell could be discovered. Voltage-clamp analysis shows that bursting cells have an N-shaped steady-state current-voltage (I-V) curve and that the net steady-state ionic driving forces of a pacemaker cell are such that the zero current or "resting potential" condition does not lie on one of the positive slopes, but in the negative slope region of the I-V curve. Hence the cell has no "resting" potential but oscillates between the two positive slope regions of the I-V curve.

Ionic studies show that the two positive slopes of the I-V curve result from high potassium conductances which are voltage and time dependent. The positive slope at large negative potentials is due to anomalous rectification while that at small negative and positive potentials is due to delayed rectification.

The negative slope region of the N-shaped curve is due to a voltage dependent but apparently time independent sodium conductance. This conductance appears to be highly specific for sodium ions and is regulated by divalent cations (enhanced Sr and Ba; inhibited by Ca and Co).

Significance to Biomedical Research and the Program of the Institute: Since this project is relatively new and the experiment results incomplete, it is premature to be precise or

detailed about the actual significance of this research. One can point out, however, a number of areas of its potential significance.

In the first place, the study, in time, of the development of the chick muscle cell in tissue culture may provide insight into the manner in which growth and maturation of membrane excitability occurs and the mechanisms which control these processes. Such insights would be of scientific interest in their own right, but are of potential medical interest for a rational understanding of muscular disorders which may have a developmental basis.

Second, rhythmic or pacemaker activity is a property found in a variety of excitable tissues (nerve cells, heart muscle, and smooth muscle) and improved understanding of the membrane mechanism underlying this activity would be of interest to a number of scientific and medical disciplines.

The third and perhaps most interesting area of potential significance of this research stems from the apparent similarity of the membrane mechanisms with different ionic bases underlying two quite distinct physiological processes in completely different tissues, viz. 1) slow contractions in immature chick muscles and 2) periodic oscillations in the membrane potential of a snail cell that controls the release of hormones. Electrophysiologists have heretofore concentrated mainly on the "fast" processes of excitable tissues and have found a considerable ubiquity in nature in the membrane mechanism which underlies these "fast" processes. Many excitable tissues have, however, a variety of "slow" processes which appear to be important in the regulation of physiological activity. It would, therefore, be of considerable interest to know if there were also an ubiquity in nature in the membrane mechanisms underlying these "slow" processes.

Proposed Course of the Project: It is proposed to continue the research on both chick muscle in tissue culture and on the snail cells by means of the voltage-clamp technique. One aim will be a more detailed documentation on the time course of development of the excitable membrane of the chick muscle. In addition, a more complete analysis of the regulation of the time- and voltage-dependent ionic conductances underlying the "slow" processes in both the chick muscles and the snail neurons will be attempted. As pointed out in the project report "Photoreceptors in the Limulus eyes" (NDS(I)-65 LNP/SP-1239), where the emphasis has been on the slow and steady state properties of those photoreceptors, one finds an N-shaped I-V curve. Moreover, the photoreceptor's I-V curve and its excitability are altered by ionic and pharmacologic manipulations that are

similar to those observed in the snail pacemaker neuron. Since it appears that similar membrane mechanisms may be involved in these two distinct cellular types, the Limulus photoreceptor project will be cancelled as an independent project and future work on Limulus included in this project.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/CB-1659
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Synaptic Contacts of Visual Cells

Previous Serial Number: Same

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: Pier Lorenzo Marchiafava, M.D.
Silvana Vallerga, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	2.0
Other:	0.5

Project Description:

Objectives: To investigate the fine structure and function of receptors and synapses in the retina.

Methods Employed: Electron microscopy combined with silver impregnations by the method of Golgi. Electrical recordings with intracellular microelectrodes.

Major Findings: The input resistance of the photoreceptor cells in the salamander retina was calculated from the voltage displacement induced by constant current pulses in darkness or at the peak of the photoresponse.

In darkness the input resistance of the rod cells is time- and voltage-dependent, while cone cells show approximately linear voltage-current relations. Following illumination, the input resistance of rods decreases while, as reported by previous authors, the input resistance of cones increases.

These results support the current view that the cone photo-response is the consequence of a reduction in the permeability of channels which in darkness shunt the membrane. In rods, however, it appears that the main result of illumination is to increase the permeability of the membrane to ions for which the equilibrium potential is more negative than the membrane potential in darkness.

The synaptic actions on salamander photoreceptors have also been studied. Rods responses are facilitated by illumination of the near surround (circle of not more than 50μ radius) while illumination of areas beyond a radius of 50μ has no significant effect on the response. In contrast, cone responses are not facilitated by the near surround, but are inhibited by illumination of the distant surround. Thus, it seems that rods are coupled to neighboring receptors, either directly or through the mediation of other retinal neurons, while cone cells are the only receptors receiving a feed-back signal from horizontal cells.

Significance to Biomedical Research and the Program of the Institute: It is hoped that these observations will help in identifying the mechanisms of synaptic transmission between photoreceptor cells and second order neurons, and provide a better knowledge of the neuronal networks involved in the processing of visual information within the retina.

Proposed Course of the Project: The synaptic actions on the photoreceptors will continue to be studied, in particular with the aim of determining whether there are interactions between rods and cones. The properties of the synapses between photoreceptors and horizontal cells will be investigated by analyzing the characteristics of the horizontal cell responses.

Honors and Awards: None

Publications:

Lasansky, A., and Marchiafava, P.L.: Light-induced resistance changes in retinal rods and cones of the tiger salamander, J. Physiol., 236: 171, 1974.

Lasansky, A.: Synaptic actions on retinal photoreceptors: morphological aspects. Fed. Proc., 33: 1069-1073, 1974.

Serial No.:NDS(I)-73 LNP/CB-2016
1. Laboratory of Neurophysiology
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Synaptic Organization of the Inner Plexiform Layer of the Larval Salamander Retina

Previous Serial Number: Same

Principal Investigator: Margaret T.T. Wong-Riley, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: To investigate the organization of the inner plexiform layer of the salamander retina, and to describe the synapses between bipolar, amacrine and ganglion cells.

Methods Employed: Eye cups of larval salamander were excised and fixed for either light (Golgi-impregnation) or electron microscopic examination. Serial sections were taken from different animals, and individual processes were traced, whenever possible, to their cells of origin. From the serial sections the consistent morphological characteristics of the bipolar and amacrine processes and ganglion cells dendrites were determined, and the synaptic interconnections between the three types of cells were studied.

Major Findings: Amacrine processes contain a fairly uniform population of small round vesicles and make conventional synapses of the symmetrical type. Bipolar processes contain vesicles that are generally larger and more pleomorphic than those of the amacrine processes, and they make ribbon synapses as well as conventional synapses of the asymmetrical type. Amacrine processes form synapses with other amacrine processes, and also with bipolar and ganglion cells. Amacrine cells also give rise to somatodendritic synapses. Bipolar processes are presynaptic on amacrine processes, ganglion cell dendrites as

well as other bipolar processes, but never on the somata of any cell. Both amacrine and bipolar processes are engaged in serial synapses, and these two groups often make reciprocal synapses with each other.

Significance to Biomedical Research and the Program of the Institute: The synaptic interconnections between bipolar, amacrine and ganglion cells found in this study provide the morphological basis for future electrophysiological studies in the inner plexiform layer of the retina.

Proposed Course of the Project: Due to the departure of Dr. Wong-Riley this project has been terminated.

Honors and Awards: None

Publications:

Wong-Riley, M.T.T.: Synaptic organization of the inner plexiform layer in the retina of the tiger salamander. J. of Neurocytology. (in press), 1974.

Serial No.:NDS(I)-71 LNP/GP-1889
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Agents Affecting Cell Proliferation and Differentiation in the Crystalline Lens

Previous Serial Number: Same

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia A. Grimes, B.A.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	1.8
Professional:	0.9
Other:	0.9

Project Description:

Objectives: To define the mechanism by which the beta-adrenergic agonist, isoproterenol, inhibits mitosis in lens epithelium and to determine the function of cyclic AMP in mediating this possible hormonal regulation of cell proliferation in the lens.

Methods Employed: Lenses, carefully isolated from young rats were maintained in vitro in small Petri dishes containing 4 ml of medium 199 supplemented with additional bicarbonate buffer and calcium chloride. The lenses were incubated at 37°C in equilibrium with a gas phase of 95% air and 5% carbon dioxide. After appropriate intervals the lenses were examined for clarity and fixed; mitotic activity and the number of cells synthesizing DNA were determined by methods previously described.

Major Findings: Mitotic activity was inhibited after two hours of culture with IPR at concentrations between $5x10^{-5}$ and $5x10^{-6}$M, but after four hours the number of mitosis returned to the normal value. Twenty-four hours of exposure to the drug-containing medium resulted in only marginal reduction of cell

division except at the highest concentration tested ($1x10^{-4}M$), at which level, IPR caused widespread cell death in the epithelial layer. The transient suppression of mitosis after two hours of incubation with IPR could be completely blocked by propranalol demonstrating that the catecholamine-induced mitotic inhibition occurred through stimulation of beta-adrenergic receptors in the isolated lens. Although the inhibitory action of IPR was no longer evident after four hours of incubation, addition of the drug to lenses which had been preincubated for two hours in control medium resulted in suppression of mitosis one and two hours later. The lens, therefore, remained sensitive to the catecholamine between two and four hours of culture, but the mitotic inhibition, once triggered, manifested itself rapidly and was transient in nature.

The slight reduction of mitotic activity seen after 24 hours of incubation with IPR was attributed to cumulative non-specific or toxic action of the drug or its degradation products, because there was no inhibition of the DNA synthesizing phase of the cell cycle at any interval preceding the mitotic depression.

The monobutyryl and dibutyryl derivatives of cAMP ($1x10^{-3}M$) mimicked the action of IPR in inducing early transient suppression of mitosis. The effects of these agents were somewhat more prolonged than that of IPR, and, besides inhibiting the number of cells entering mitosis, the cAMP derivatives at high concentration directly affected the mitotic process. After two hours of culture, entry into mitosis was almost completely blocked, but an abnormal accumulation of metaphase and telophase figures at this interval indicated preceding or concurrent metaphase arrest. The shift in phase counts was of shorter duration than the mitotic inhibition for at three and four hours of incubation all phases of mitosis were equally suppressed. Mitotic inhibition in turn was no longer evident after six hours of incubation, and in experiments with MBcAMP no further changes in mitotic activity occurred. DBcAMP, in contrast, caused a second period of metaphase arrest at 16 and 24 hours of incubation. This late effect on the mitotic process was equally evident when lenses were exposed for similar periods to sodium butyrate alone ($1x10^{-3}M$), although this compound did not evoke the early changes in mitotic activity seen with cAMP derivatives. It seems probable that the interference with mitotic division seen after prolonged incubation with DBcAMP resulted from accumulation of butyrate in the medium.

Significance to Bio-medical Research and the Program of the Institute: The clarification of the effects of these agents on the non-vascularized, non-innervated lens epithelium may contribute to the understanding of the complex mechanisms governing the control of proliferation and differentiation in cell populations of different functional properties.

Proposed Course of the Project: Studies will be continued to search for possible intermediary agents involved in the IPR-induced suppression in mitosis such as prostaglandins, and if possible, to provide a link between the different inhibitory phenomena observed *in vivo* and *in vitro*.

Honors and Awards: None

Publications:

von Sallmann, L. and Grimes, P.: Effects of isoproterenol and cyclic AMP derivatives on cell division in cultured rat lenses. *Invest. Ophthal.* 13: 210, 1974.

Serial No.:NDS(I)-72 LNP/GP-1943
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Spontaneous Retinal Degeneration in Osborne-Mendel Rats

Previous Serial Number: Same

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia A. Grimes, B.A.

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To study the development of a newly-discovered retinal degeneration in mature Osborne-Mendel rats, and to compare this progressive retinal destruction with a possibly similar degeneration reported to occur in a strain of spontaneously hypertensive Wistar rats.

Methods Employed: The presence and extent of retinal degeneration was determined by histological examination of rats of various ages from four colonies maintained by the NIH Animal Production Unit: inbred Osborne-Mendel (OM/N), non-inbred Osborne-Mendel (OM), spontaneously hypertensive Wistar (SHR/N), and non-hypertensive Wistar (WSKY/N).

The spontaneously hypertensive animals bred at NIH originated directly from the line of hypertensive Wistar rats developed in Japan. The non-hypertensive Wistar rats descend from the original Wistar colony at Kyoto University from which the SHR were isolated.

Major Findings: Our original observations of retinal degeneration in mature Osborne-Mendel rats was based upon the examination of 28 animals between the ages of 7 and 13 months, of which 18 showed destruction of photoreceptor cells in varying severity. We have since examined an additional 67 rats (6 months-2 years old)

from the Osborne-Mendel colonies and have found the incidence of degeneration in this larger sample to be approximately the same as that noted previously (73%). Because of disparities in sample size at different ages, it was difficult to relate the presence or severity of degeneration to age. However, it was evident that some animals showed signs of degeneration by 6 months of age, and that normal rats persisted in the population to the age of 15 months. At any given age the severity of the disease varied widely, suggesting that the time course of the disease was not constant from animal to animal. Total loss of the photoreceptor layer occurred as early as 9 months in some individuals, while other animals of the same age or older showed only early stages of retinal degeneration.

Of 13 SHR/N rats examined between the ages of 10 and 15 months, 8 had retinal degeneration. The prevalence of the disease in this colony was similar to that reported for the original Japanese stock (2 of 3) as well as to that characteristic of the OM and OM/N colonies. Retinal degeneration has not been detected in WSKY/N rats previously, but we found one of 5 examined animals to be affected. The number of animals studied were too small to permit any estimation of frequency in this strain. No differences in the histopathologic development of the degeneration among the colonies was seen with the light microscope. All of the affected animals showed more or less advanced stages of a degenerative process initiated by disintegration and disappearance of outer segments and progressing to destruction of all photoreceptor elements without any accumulation or persistance of outer segment material. There was no indication that the hypertensive state contributed to the retinal degeneration in SHR rats. The only ocular sign of hypertension was a significant narrowing of retinal arterioles which was demonstrated in trypsin-digested histological preparations of the retinal vessels in all SHR rats, regardless of the presence or absence of retinal degeneration.

Significance to Biomedical Research and the Program of the Institute: The retinal degeneration observed in Osborne-Mendal rats resembles certain human retinal degenerations more closely than do other types of animal retinal dystrophies. It may provide a valuable model for identification of the cause of photoreceptor degeneration in such conditions.

Proposed Course of the Project: We will continue to study the rat colonies affected with retinal degeneration to evaluate the influences of genetic and environmental factors in development of the disease. A large sample of the WSKY/N rats will be examined to determine the incidence of degeneration in this colony which in other respects serves as an effective control strain for SHR/N rats.

Honors and Awards: The Howe Prize Medal in Ophthalmology was presented to Dr. vonSallmann in recognition of the excellence of his research.

Publications: None

Serial No.:NDS(I)-72 LNP/GP-1944
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Organization of a Molluscan Central Nervous System

Previous Serial Number: Same

Principal Investigator: Daniel L. Alkon, M.D.

Other Investigator: Anthony Bak

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.3
Other:	0.0

Project Description:

Objectives: The principal objective is to understand synaptic mechanisms underlying the interaction of sensory pathways in the primitive nervous system of Hermissenda crassicornis, a nudibranch mollusc.

Methods Employed: Intracellular recording from single hair cells, photoreceptors and visual second-order cells of Hermissenda crassicornis has been used.

Stimulation of the hair cells is accomplished with an electrostrictive device which causes controlled and reproducible displacement. The displacement produces hair cell responses without appreciably disturbing the intracellular electrode.

A new stimulus for the hair cells, orientation change, was provided by the use of a "tilt table" which also allows simultaneous intracellular recordings.

Major Findings: It was seen that hair cells receive synaptic input from the visual pathway and that photoreceptors are influenced synaptically by hair cell activity. The synaptic organization underlying this interaction appears rather specific. There is little convergence for any one type of ipsilateral visual synaptic input to hair cells although a given hair cell may be

affected at the same time by different types of synaptic input such as a Type A and Type B photoreceptor, an ipsilateral optic ganglion cell and a contralateral photoreceptor.

Two principal hair cell light responses were found:

(1) hyperpolarization and cessation of firing,
(2) hyperpolarization followed by depolarization and increased firing.

Using a small spot of light to illuminate each eye separately it could be demonstrated that the depolarizing visual input to hair cells arises largely from the ipsilateral eye. The hyperpolarizing input arises from both eyes, but more frequently from the contralateral eye.

Progress in understanding the synaptic interaction of the visual and statocyst pathways, as well as the functional significance of this interaction, was made with three experimental approaches:

(1) Simultaneous intracellular recording from photoreceptors and hair cells;
(2) the use of a "tilt table" which makes possible changes in orientation of the statocyst while recording intracellularly from hair cells;
(3) modification of the animals' behavior with associated visual and rotational stimuli and correlating electrophysiologic changes with the behavioral modification.

With the first technique new insight was gained into the mechanism by which the depolarizing visual input to hair cells arises. It was found that ipsilateral Type A photoreceptors excite hair cells by causing a decrease in conductance. This excitation could be maintained as long as the Type A photoreceptor was excited by intracellular depolarizing current. Type A photoreceptors were also frequently observed to briefly hyperpolarize the same hair cell it excites. This dual synaptic influence on hair cells from Type A photoreceptors will be the subject of future study.

Using the "tilt table" it was found that hair cells depolarize in response to a change in orientation in a direction toward the locus of the hair cell on the statocyst. Hair cells hyperpolarize (with an associated decrease in impulse in activity), in response to a change in orientation in a direction away from the locus of the hair cell on the statocyst. The threshold stimulus for these responses ranged from one to two degrees. The responses were also found to be graded with the number of degrees of orientation change.

Finally, a training regimen was developed in which visual stimulation paired with rotation produced a behavioral modification (for approximately 1 1/2 hours) not produced by either type of stimulation when not paired with the other. The depolarizing response of hair cells to stimulation of the ipsilateral eye was observed to be substantially reduced or abolished (for approximately 1 1/2 hours) in those animals which had been exposed to the associative training. The hyperpolarizing response of hair cells to stimulation of the contralateral eye was observed to appear less frequently in animals given the same training. Additional observations indicated that these electrophysiologic changes resulted from synaptic changes and not changes in the responses of the photoreceptors to light or in the membrane properties of the hair cells.

Significance to Biomedical Research and the Program of the Institute: The visual system and statocyst system (analogous to mammalian vestibular systems) may be the first preparation where definition of intersensory information exchange is possible. Such definition might contribute to our understanding of the visual and vestibular processes and their interactions as well as to neural organization in general.

Proposed Course of the Project: Work with the "tilt table" will be continued to gain further knowledge of how the statocyst responds to orientation changes, and how these responses affect and are affected by synaptic input from the visual system. An additional stimulus, a turn table, will be used to examine in a similar way the response of the statocyst to centrifugal acceleration. Further electrophysiologic and histologic analyses are planned to more precisely understand the synaptic connections mentioned above.

Honors and Awards: None

Publications:

Alkon, D.A.: Neural organization of a molluscan visual system. J. Gen. Physiol. 61: 444-461, 1973.

Alkon, D.A.: Intersensory interactions in Hermissenda. J. Gen. Physiol. 62: 185-202, 1973

Alkon, D.A. and Bak, A.: Hair cell generator potentials. J. Gen. Physiol. 61: 619-637, 1973.

Serial No.:NDS(I)-72 LNP/GP-1945
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Neural Organization of the Vertebrate Retina

Previous Serial Number: Same

Principal Investigators: Elliott J. Simon, M.D.
Amrei Richter, Ph.D.

Other Investigators: M.G.F. Fuortes, M.D.
Paul O'Bryan, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.3
Professional:	2.3
Other:	0.0

Project Description:

Objectives: This project is concerned with the elucidation of mechanisms by which electrical responses to light are processed and modified in the vertebrate retina.

Methods Employed: The retina of the turtle has been chosen as the experimental preparation because the anatomy of its synaptic connections has been carefully described by other investigators and because its cells are large enough to permit intracellular recording with microelectrodes. Neurons (primarily photoreceptors and horizontal cells) are impaled with microelectrodes and their responses to different light stimuli are recorded. Identification of the cells from which electrical responses originate is determined by intracellular marking by dye iontophoresis.

Major Findings: Cone photoreceptors in the turtle retina contain visual pigments which maximally absorb either red, green or blue light. Absorption of light by a cone elicits in it a slow hyperpolarizing response which is graded with light intensity ("direct response"). Stimuli of any color which cover small areas produce the same direct response in single cones if their intensities are adjusted appropriately. In double cones,

however, the responses of red-sensitive and green-sensitive receptor elements are coupled; therefore, the direct response of these cells varies with stimulus color.

Luminosity horizontal cells ("L-cells") are synaptically connected to the photoreceptors and to other L-cells; L-cells respond to large areas of retinal illumination due to electrical coupling between them. L-cells feed a depolarizing wave back onto cones of all spectral sensitivities when the illuminated field is large; this wave combines with the direct response to give a composite cone response. The input to these L-cells is primarily from red-sensitive cones but also includes double cones; consequently, the feedback component of the composite cone response has a mixed color input. Because of this organization the net cone response differs for different colors of stimulation even when the direct responses are identical. Depending on the color, intensity, and pattern of illumination the cone response varies in size, shape, or polarity.

We have also found that there are horizontal cells connected primarily to green-sensitive cones (green/blue chromatic cells). These cells further interact with one another and with the L-cells producing net responses which contain information about all aspects of a visual stimulus--color, intensity and pattern.

Significance to Biomedical Research and the Program of the Institute: The retina is one of few parts of the vertebrate central nervous system which is simple and accessible enough to permit the detailed cellular investigations necessary to determine fundamental principles of organization of the CNS. The input (light stimuli) and the output (optic nerve activity) of the retina can be fully determined. All processing which transforms the light into the complex neural codes of the output is performed by only five cell types all of which can be studied by intracellular recording. This project is designed to elucidate the mechanisms by which the various retinal cells interact to process visual information.

Proposed Course of the Project: Current and future investigations include further study of the interactions of first order (receptors) and second order (horizontal cells and bipolar cells) neurons which will permit a detailed description of the electrical circuitry of the outer part of the retina. In particular, investigations will be performed to determine how the responses of the different horizontal cells interact with one another and with receptors and how they combine to generate responses in bipolar cells, which are the only link to third order retinal neurons.

Honors and Awards: None

Publications:

Fuortes, M.G.F., Schwartz, E.A. and Simon, E.J.: Colour-dependence of cone responses in the turtle retina. J. Physiol. 234: 199-216, 1973.

Fuortes, M.G.F. and Simon, E.J.: Interactions leading to horizontal cell responses in the turtle retina. J. Physiol. 1974 (in press).

O'Bryan, P.M.: Properties of the depolarizing synaptic potential evoked by peripheral illumination in cones of the turtle retina. J. Physiol. 235: 207-223, 1973.

Simon, E.J.: Feedback loop between cones and horizontal cells in the turtle retina. Fed. Proc. 33: 1078-1082, 1974.

Serial No.:NDS(I)-73 LNP/GP-2017
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechano-Transduction in Invertebrate Hair Cells

Previous Serial Number: Same

Principal Investigator: Peter Detwiler, Ph.D.

Other Investigator: M.G.F. Fuortes, M.D.

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	2.5
Other:	0.0

Project Description:

Objectives: To study the kinetics of mechano-transduction in the hair cells of the invertebrate Hermissenda crassicornis. The project consists of two parts. First to describe the responses to experimental stimuli and the changes induced by temperature, ion substitution and metabolic poisons.

Methods Employed: Hair cells in the statocyst (a primitive vestibular apparatus) of Hermissenda were stimulated with a piezoelectrically driven stylus, and their responses were recorded by means of intracellular microelectrodes.

Major Findings: Hair cells have a resting membrane potential of approximately 45 mV. They are usually spontaneously active with impulses occurring at a rate of 3-4/sec. The average input resistance of the cell is 125megΩ. Charging curves produced by a step of constant current have two time constants (5 and 50 msec). Hair cells respond to mechanical stimulation with a depolarizing generator potential. The response has a latency of 2 to 3 msec., reaches peak in about 15 msec and decays with a time constant of 50 to 100 msec. The relation between response amplitude and displacement of the statocyst was determined by simultaneous stroboscopic photography and intracellular recording. The results revealed that the amplitude of the response (when corrected for reversal potential proximity) grows more than in proportion with the displacement of the statocyst. The hair cell response

reverses polarity on the positive side of the resting potential. Unfortunately, technical difficulties made direct measurement of the reversal potential impossible. Reversal potential was therefore estimated by extrapolating the current voltage relationships of a hair cell before and at the peak of the generator potential. In so doing it was found that the reversal potential varies with stimulus strength. Weak stimuli evoke responses with an infinite reversal potential. With stronger stimuli reversal occurs at highly positive potentials and approaches zero membrane potential as the stimulus strength is increased further.

Decreasing the temperature by 5 to 10°C increases the latency, the time to peak and the decay time of the response. The effects of ion substitution, and metabolic inhibitors have, thus far, only been studied in a preliminary way.

The above results may be explained by assuming that the site of mechano-transduction is on the cilia and that as the stimulus strength is increased the transduction process involves a greater portion of the total ciliary membrane. The response shape and its temperature dependence is fit by an equation that describes, as a function of time, a series of five transformations. The results are, thus, consistent with the notion that mechano-transduction involves a succession of steps which are triggered by deformation of the ciliary membrane and are sensitive to temperature.

Significance to Bio-Medical Research and the Program of the Institute: Mechano-receptors are probably the most ubiquitous sensory receptor in the animal kingdom. In higher organisms they provide information about a greater spectrum of stimuli than any other single receptor. In man, for example, mechano-receptors are responsible for the skin sensations of touch and pressure, for muscle and internal organ proprioception, for joint position sense, for gut reflexes that give rise to peristalsis, for cardio-vascular reflexes dependent upon blood pressure, for hearing, and for the senses of balance, acceleration, and direction of gravity. In spite of its widespread distribution and fundamental importance mechano-reception is a poorly understood process. It is hoped that the present study will provide some useful insight on how they function.

Proposed Course of the Project: During the next year the experiments proposed in this report will be completed and the data published.

Honors and Awards: None

Publications:

Detwiler, P.B. and Alkon, D.L.: Hair cell interactions in the statocyst of Hermissenda. J. Gen. Physiol. 62: 618-642, 1973.

Serial No.:NDS(I)-73 LNP/GP-2018
1. Laboratory of Neurophysiology
2. Section on General Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Ionic and Pharmacologic Basis of the Inhibitory Interaction Between Photoreceptors of a Simple Visual System

Previous Serial Number: Same

Principal Investigator: Leona M. Masukawa

Other Investigator: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The objective of this project was to understand mechanisms of synaptic action on the photoreceptors (both excitatory and inhibitory) and to describe the ionic permeabilities of the photoreceptor membrane.

Methods Employed: Experiments were performed using the photoreceptor cells of Hermissenda crassicornis. Intracellular measurements of membrane potential were made during alterations of the ionic composition of the bathing medium. Membrane potential was displaced by extrinsic currents to determine current-voltage relations at rest and during activity.

Major Findings: The photoreceptor membrane is almost impermeable to chloride ions. In high concentrations of potassium the membrane acts like a potassium electrode (i.e., has a high potassium permeability). At normal potassium concentrations (10-20mM), however, the permeability to potassium is low.

The reversal potentials for the after-hyperpolarization of the spike and the IPSP were found to be similar. This is consistent with the finding that the IPSP on the potassium ion concentration.

However, during chloride-free conditions (acetate substitution inhibitory interactions evoked by electrical presynaptic stimulation or light flashes disappeared, and reappeared when chloride was returned to the bathing medium. These findings indicate either the influence of either chloride or acetate on release of transmitter or transmitter receptor interaction.

Excitatory postsynaptic potentials are also seen during intracellular recordings. These EPSP's are associated with increased membrane conductance and are blocked, along with the evoked IPSP's by Cobalt ions (10 mM). They appear to be a source for the spiking seen in the dark adapted photoreceptor. This excitatory input represents a new pathway to modulate the activity of the photoreceptor. EPSP's are blocked by curare and atropine at concentrations that do not block IPSP's.

Significance to Biomedical Research and the Program of the Institute: Because of the simplicity and accessibility of the visual system of Hermissenda, research can be carried out which can aid in the understanding of more complex and technically inaccessible visual systems and synapses.

Proposed Course of the Project: This project will be terminated.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-71 LNP/NI-1892
1. Laboratory of Neurophysiology
2. Section on Neuronal Interactions
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative Physiology of Invertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: John S. McReynolds, M.D.

Other Investigator: Anthony L.F. Gorman, Ph.D.

Cooperating Units: Department of Neurobiology, Marine Biological Laboratory Woods Hole, Massachusetts

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description:

Objectives: The overall objective of this project is to study the mechanisms by which receptor neurons convert external stimuli into the electrical signals which are transmitted to higher centers in the nervous system. In particular, the present work is concerned with the control of membrane permeability and membrane potential of the primary sensory neuron by the stimulus.

Methods Employed: Intracellular recording with glass microelectrodes were used to measure the membrane potential of single receptor neurons in the retina of the mollusc, Pecten irradians. Previous work, described in earlier annual reports on this project, has revealed that this preparation has a number of features which make the study of these receptors of special interest. Measurements of membrane resistance under different conditions were also made. Continuing the previously reported work, the effect of specific changes in the extracellular ionic medium were studied. Also, the effect of various metabolic inhibitors on membrane potential and permeability were investigated.

Major Findings: This work has established that the primary electrical response (the receptor potential) in these sensory neurons to the light stimulus is caused by a selective increase

in potassium permeability of the cell membrane. Studies with metabolic inhibitors indicate that there is also a link between cellular metabolism and the membrane permeability to potassium: the inhibitors 2,4-dinitrophenol and cyanide increase potassium permeability and hyperpolarize the cell. These findings suggest that potassium permeability (and therefore the membrane potential) is maintained at the "resting" level by an active metabolic process.

In other neurons it has been shown that potassium permeability may be increased by a rise in intracellular concentration of calcium ions, and also that the metabolic inhibitors 2,4-DNP and cyanide may lead to an increase in intracellular Ca^{++}. Experiments were performed to test the hypothesis that an increase in intracellular Ca^{+2} might be one step in the process by which light normally controls membrane K^{+} permeability in these photoreceptors. Experiments involving alteration of external Ca^{+2} concentration have so far been inconclusive due to inability to maintain recording in the presence of altered external Ca^{++}. However, it was found that low concentration of Cobalt (Co^{++}) and magnese (Mn^{++}), which are known to interfere with Ca^{+2} entry at presynaptic terminals, significantly reduced the light induced hyperpolarization. This suggests that entry of Ca^{+2} from outside the cell may be a part of the generation of the receptor potential.

Significance to Biomedical Research and the Program of the Institute: Although a great deal has been learned about the initial photochemical reactions in visual receptors, and about the changes in membrane permeability which generate the sensory neuron's electrical signal, very little is known about the mechanisms which control membrane permeability of sensory receptors. This gap applies to our knowledge of the visual system. The findings that the receptor potential may be generated by a selective increase in potassium permeability, and that membrane permeability may also be regulated by metabolic activity, may be important to our understanding of how sensory neurons function.

Proposed Course of the Project: This project is to be terminated.

Honors and Awards: None

Publications:

McReynolds, J.S. and Gorman, A.L.F.: Ionic basis of hyperpolarizing receptor potential in scallop eye: increase in permeability to potassium ions. Science. 183: 658-659, 1974.

Serial No.:NDS(I)-71 LNP/NI-1895
1. Laboratory of Neurophysiology
2. Section on Neuronal Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Microspectrophotometric Studies of Vertebrate Photoreceptors

Previous Serial Number: Same

Principal Investigator: Ferenc I. Hárosi, Ph.D.

Other Investigator: Franco Malerba, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The aim of this project is to reveal the spectroscopic properties of visual pigments of vertebrate photoreceptors in situ.

Methods Employed: Freshly isolated rod and cone cells obtained from dark-adapted retinas are suspended in solutions of various composition. Thin preparations of such suspensions are used to measure single cells in a microspectrophotometer. Absorption spectra are recorded and linear dichroism is measured in the dark and following brief flashes of bright light. The formation and decay of photoproducts are measured in terms of spectral properties and dichroism under selected ionic conditions, at +5 to +25°C.

Major Findings:

1) Salamander rods: According to the literature (Liebman, 1972, Handbook of Sensory Physiology, VII/1, p. 507), the tiger salamander is supposed to have visual pigments like the leopard frog. Preliminary results obtained from salamander rods indicate this not to be the case. The visual pigment of these cells appears to be a mixture of rhodopsin and porphiropsin for the following reasons: a) the main absorption band has λ_{max} = 515 nm; b) the absorption of the β band peaks at about 395 nm; c) the

main band is broader than that of the rhodopsin-type pigments; d) the transverse dichroic ratio is about 3.6:1, which is smaller than that of frog rods (4-5:1) but larger than that of goldfish cones (2-3:1).

2) Theoretical Studies: The absorptive interaction between plane-polarized light and linear transition moment of chromophores of side-illuminated, single, cylindrical photoreceptors were studied. The results show that the numerical aperture of the condenser is an important parameter in microspectrophotometric measurements. The analysis makes it possible to interpret with confidence the experimentally determined dichroic ratios in terms of the fraction of extinction that is displayed in the direction parallel with the long axis of the cell. It is shown that for small apertures ($NA \leq 0.4$) usual assumption of collimation is tenable. For larger apertures this condition does not hold but accurate calculations can be performed and it is possible to interpret the results obtained with any size of aperture.

3) Hypothesis to account for transverse dichroism of photoreceptors: The experimental findings can be understood assuming that the orientation of retinal (1 and 2) is precise in photoreceptors; it is such as to maximize absorption in the physiological direction. As a consequence of this orientation, most of the conjugated double bonds are found in planes of the transverse discs, except those of the six-membered ring. Absorption of light by the ring portion of conjugation may in turn account for the transverse cell dichroism. It is shown that the model is consistent with data of 1) transverse dichroism obtained from all photoreceptors; 2) x-ray data of 11-cis and all-trans retinals in crystals; 3) theoretical calculations on the spectral characteristics of the retinal isomers; 4) the dichroic ratio of dark-adapted frog rods, goldfish cones, salamander rods; 5) dichroic ratio of bleached goldfish cones; 6) dichroic ratio of bleached frog rods with intermediates of a) metarhodopsin II, b) metarhodopsin III, c) retinol, d) retinal oxime.

Significance to Biomedical Research and the Program of the Institute: The twofold aim of this project, namely 1) to characterize the absorptive properties of vertebrate photoreceptors and 2) to understand the photo-chemical process of bleaching in visual pigments, are important both for the interpretation of electrophysiological results and for the elucidation of the molecular mechanisms underlying vision in vertebrates including man.

Proposed Course of the Project: To continue the study of 1) mudpuppy rods and cones 2) salamander rods and cones, 3)

goldfish cones, 4) turtle cones, 5) pigment regeneration in isolated visual cells of mudpuppy and goldfish with 11-cis retinal$_1$. These are necessary to further elucidate the basis of the two hypotheses: one, about the orientation of the chromophores (above), and one (proposed last year) concerning the invariance of oscillator strength of the main absorption bands of the porphyropsin-type cone pigments to account for the variation of spectral width and molar extinction. 6) It is also planned to conduct chemical studied (with hydroxylamine, methoxyamine, etc.) aimed at gathering information about the active site of visual pigments and the mechanism of photo-excitation.7)I also intend to continue the study of cone morphology so that we may establish their connections to other cells in the goldfish retina (in collaboration with W.K. Stell).

Honors and Awards: None

Publications:

MacNichol, E.F. Jr., Feinberg, R. and Harosi, F.I.: Color discrimination processes in the retina. In Colour 73, p. 191-251. Proceedings for the Second Congress of the International Colour Association. Adam Hilger, Rank Precision Industries, Ltd., London, 1973.

Harosi, F.I. and MacNichol, E.F. Jr.: Visual pigments of goldfish cones. Spectral properties and dichroism. J. Gen. Physiol. 63: 279-304, 1974.

Annual Report
July 1, 1973 through June 30, 1974
Laboratory of Biophysics, IR
National Institute of Neurological Diseases and Stroke

William J. Adelman, Jr., Ph.D., Chief

Introduction

The overall research program of the Laboratory of Biophysics has been concerned with uncovering basic molecular and cellular mechanisms associated with the generation of the nerve impulse and of synaptic activity. This program has involved the study of membrane excitability and neural function in natural excitable membranes in both nerve and muscle and in artificial membrane systems. The Laboratory has made wide use of physical and chemical techniques, on-line and off-line digital computers, and a variety of applied mathematical methods. The most interesting aspect of this program has been a convergence of interest on ion movements through individual molecular channels in natural and artificial membranes. The Laboratory has continued to expand its studies on both intracellular and extracellular sub-structures in nerve and muscle tissue. Understanding the role of these adjunct structures with respect to membrane function will expand our understanding of nerve and muscle. The Laboratory has established collaboration with numerous universities and institutes. Of especial interest has been the collaboration with Carnegie-Mellon University, The University of Cincinnati, State University of New York at Albany, The University of Rochester, The Technion-Israel, and the marine biological laboratories in Woods Hole, Mass., Plymouth, England and Montemar, Chile. Invited review lectures have been given by staff members in the Colloquium on "Membranes, Ions, and Impulses" at the Federation Meeting in Atlantic City, N. J., in the Symposium on the Neurosciences at MIT, and in the Excitable Membrane Program at the Marine Biological Laboratory. One major review on excitable membranes will appear in the "1974 Annual Review of Physical Chemistry" and invitations have been accepted to prepare reviews for "Progress in Neurobiology" and "Current Topics in Membranes and Transport". Two staff members have received an invitation to prepare a book on "Biophysical Methods". The following are some highlights of an exciting year of research:

Voltage Clamp Studies on Membrane Conductances in Giant Axons

Four different squid giant axons (from Loligo pealei, Loligo forbesii, Loligo vulgaris and Dosidicus gigas) and a polychaet marine worm giant axon (from Myxicola infundibulum) have been studied. These studies have examined in detail the effects of diphenylhydantoin (DPH) on the kinetics of sodium channel conductance, the permeability of sodium channels to divalent ions, and the action of veratridine on sodium and potassium ion conductances. These studies are providing us with insights into the molecular nature of the ionic channels associated with excitability and with a basis for the action of neuro-pharmacological agents at the membrane level. The most important advance in the voltage clamp technique has

occurred through on-line data acquisition methods. Since experiments are now performed under computer control and data is recorded in digital form, data reduction and analysis have been expanded by orders of magnitude. This has made possible the testing of sophisticated models. Plans are now being made to test the two barrier, one site model (see Mathematical Modeling below) on the squid giant axon. In addition, we plan to use an on-line data acquisition system to obtain complete ionic conductance parameter values so as to account for both repetitive neuronal firing and adaptation.

On-Line Data Acquisition by a Digital Computer System

Two years ago a large data acquisition and processing system was installed in the Laboratory. This system consists of a Digital Equipment Company PDP-11/20 computer with disk and magnetic tape operating systems. Considerable software was been generated for voltage clamping, control, processing and preliminary analysis. Hardware interfaces have been designed and built for control and acquisition purposes. This system is presently being interfaced with the remote facilities of DCRT's PDP-10 for access to large computer facilities. As a result of this effort this system has been integrated with several of the on-going experimental programs in the Laboratory. At present this system is used on-line in the following experiments: (1) acetylcholine (ACh) activated post-synaptic membrane channel kinetics by shot noise analysis and (2) ionic selectivity sequences in axon membrane channel conductances. In addition, plans are being made to use the system in experiments on divalent ion channel kinetics in muscle fiber membranes.

Ionic Channels in Post-Synaptic Membranes

Two systems have been studied: (1) Acetylcholine activated channels in tissue cultured muscle cells (2) Ionic channels in the post synaptic membrane of the squid giant synapse. The object of the first of these studies was to observe single channel currents in living cells. Cultured muscle cells were chosen because they were devoid of connective tissue which could interfere with getting an electrical seal against the cell, and because the cells possessed acetylcholine sensitive channels which could be readily controlled. The first experiments did not reveal any recognizable channel responses, so it was decided to learn more about the channels using the fluctuations of many channels, and with the information thus gained, optimize the preparation and recording system for further attempts at seeing single channel responses. The cells were voltage clamped in culture using a two microelectrode point clamp, and steady doses of ACh were applied iontophoretically. Fluctuations in the output current were analyzed by frequency to form a power spectrum. The component of these fluctuations due to ACh channels was distinguished from other noise sources by subtraction of the spectrum in the absence of drug from that observed during steady state applications of the drug. The power spectrum data was fitted to a model of the channel kinetics in which the channels opened randomly (by ACh arrival) and closed randomly in a Poisson fashion. From this analysis a channel conductance of about 80pmho was derived. Such a channel carries a current of about 3pa (at normal

resting potentials), and stays open for about 1msec at 35°C. The data further suggested that the channels stay open longer with increasing membrane potentials. In the second study, experiments were performed on the squid giant synapse to determine if the post-synaptic current (PSC) is potential dependent. The declining phase of the PSC was fitted to the logistic or growth curve. The rate constant in the equation did not appear to potential dependent. Explanations for the good-fit of the data to the logistic equation are now being sought.

Synthetic Membranes

Synthetic lipid-bilayer membranes doped with various polypeptide additives serve as model systems for studying the molecular nature of membrane transport processes. The major emphasis in the Laboratory has been on two additives: (a) EIM, a protein of bacterial origin which simulates the "gating" or voltage-dependent ionic permeabilities characteristic of membrane excitation; (b) Na-K ATPase ionophore, a small protein, extracted from Na-K ATPase, which facilitates cation transport through the membrane in the presence of Na ions.

The EIM studies demonstrated the existence of discrete ionic channels, each able to transport 10^8 ions per sec in the presence of a 100 mV potential difference across the membrane. The gating process was shown to be caused by structural transitions of the ionic channels between the high and low conductance state. Electrical excitation occurs because the rate of these transitions are voltage-dependent. The evidence for the gating transitions was obtained by the novel technique introduced in the Laboratory of studying discrete electrical current fluctuations. In recent work, we have extended the study to the transport properties of individual channels so as to obtain a complete description of the gating kinetics. The transport studies demonstrated that the EIM channels form electrolyte pores through membrane, probably 15 - 20 A in diameter. The kinetic studies suggest the existence of a conformation change in which charged groups undergo rearrangements equivalent to a 600 Debye-unit dipole moment change per channel.

The Na-K ATPase studies identify a pore-forming fraction of the enzyme which is specifically activated by Na ions. The finding raises the question of whether this particular polypeptide is part of the Na-binding site of the active transport structure.

Mathematical Modeling

A new model for the sodium and potassium channels of the nerve membrane has been devised. The model uses reaction rate theory to describe a two barrier, one site channel in which ions compete for the site which can be occupied by only one ion at a time. The model predicts that permeability ratios (for different ionic species) estimated from reversal potential measurements are not the same as permeability ratios estimated from current measurements at equivalent driving forces. The model also predicts, quantitatively, deviations from the independence principle for current-voltage relations in single ion species experiments, as well as the voltage-de-

pendent action of inhibitory ions on channel conductances.

In order to increase the convenience of obtaining solutions of the Hodgkin-Huxley and other nerve membrane equations, considerable effort was expended in writing new computer programs for the PDP-10 and PDP-11. They use the numerical method of C. W. Gear, which is more economical of time than the Runge-Kutta method used previously, and also provides efficient solution of stiff equations (those with one very short relaxation time), encountered under some conditions. One program produces action potentials for various current pulse stimuli. Another computes thresholds by automatically searching for the point of maximum slope on the stimulus-response curve (peak membrane potential versus stimulus strength). This method permits a unique value of threshold to be defined, even for the graded responses which appear at high temperatures. The new computer programs for solving nerve membrane equations are being used to solve two problems, one on predictions of the Hodgkin-Huxley model modified to include potassium accumulation outside the membrane, and the other on nerve threshold behavior, especially explaining the temperature dependence of anodal break responses.

It has been found that a constant phase angle capacitor circuit with shunt resistance can be mimicked theoretically by the input impedance of a semi-infinite tapered (Bessel) transmission line. The physical model of the constant phase angle capacitance provided by the Bessel transmission line may or may not be of significance in explaining this phenomenon as observed in the nerve membrane. It provides a geometrical, macroscopic explanation, in contrast to earlier attempts to derive this phenomenon from a distribution of molecular relaxation times.

Further study of the model of optimal control of muscle is under way, for the case in which the series elastic element is not zero. Preliminary work indicates that singular perturbation theory may be useful.

An analysis has been carried out on a theory of white noise stimulation of nerve. This analysis shows that: (a) below the threshold for noise firing, the axon response can be adequately described by a slight generalization of the linearized Hodgkin-Huxley equations; (b) above threshold the statistical properties of random firing can be described by an adaptation of the Stratonovich theory of the behavior of randomly switching relays. The subthreshold analysis provides the basis for a new practical method of membrane impedance measurement.

Periaxonal Spaces and Clefts

In a collaborative study with Carnegie-Mellon University, cross and longitudinal sections through the Schwann sheath of the squid giant axon were obtained. These were examined in the electron-microsccpe. From measurements of the periaxonal space and the clefts between Schwann cells, the resistance in series with the axolemma was calculated as about $1.5 \Omega cm^2$. This value was in rough agreement with the series resistance determined from voltage clamp measurements of the external barrier permeability to potassium ions. It was found that the thickness of the anatomical periaxonal space increased about 2x when axons were exposed to 10% hypertonic sea water.

This value was also in agreement with the increase seen in the phenomenological external K^+ space obtained on voltage clamping squid axons. Therefore, it now seems that the voltage clamp measurements of the periaxonal space dimensions and the permeability of the outer barrier to K^+ ions have a real basis in the anatomical relations that satellite cells have to the axon. This agreement provided encouragement to model neuron-glial cell relationships on a similar basis to that used for modeling axon-Schwann cell relationships. Some predictions have been made which may provide an insight into the role of glial cells in epileptogenesis in the central nervous system.

In addition, an analysis of the penetration kinetics of tetrodotoxin and saxitoxin across the squid axon Schwann cell sheath has provided an explanation for the slow latency of action of these agents on the giant axon membrane.

Subcellular Structure of Nerve and Muscle

A variety of studies were carried out on intracellular water structure, cytoplasmic proteins and on the membrane forming properties of cytoplasm.

The studies on the structure of intracellular water using spin labelled single muscle fibers were extended to cover the temperature dependence of the probe in model protein solutions, and a careful analysis of the effects of fast exchange on the probe spectrum were performed. The results supported the interpretation that the probe undergoes fast exchange in the muscles between sites of different mobility, most likely between a free solution type of environment and a few relatively stiff binding sites. The viscosity values must therefore be taken as upper limits to cytoplasmic viscosity. These results indicate that cell water does not form an extensive ordered structure, and hence cannot serve as an ion exchange medium for maintaining ion balance.

In another study done in collaboration with Carnegie-Mellon University, the cytoplasm of the squid giant axon was examined for the existence of a newly discovered fibrous protein found in smooth muscle. SDS gel electrophoresis of extruded axoplasm from Loligo pealei giant axons show 12 bands. The 130,000 dalton band has small satellites on each side at approximately 150,000 daltons and 100,000 daltons. The highest concentration is at 130,000 daltons just as in smooth muscle. Amino acid composition of the material obtained from the sliced gels of each of the three bands does not correspond with any known fibrous protein, but is identical to that obtained by amino acid analysis of similar gel bands obtained from mammalian smooth muscle. It is suggested that this protein, which has been named Mesosin, is a universal fibrous protein.

A study was carried out in collaboration with the State University of New York at Albany so as to induce a "surface precipitation reaction" at the interface of amoeba cytoplasm (Chaos carolinensis), squid axoplasm (Loligo pealei) and membrane inducing solutions. A surface precipitation

reaction has been produced in demembranated amoeba cytoplasm and exuded axoplasm from the giant axon of the squid. The new surface formed when the pH, ionic strength, osmolarity, divalent cation concentration and temperature were controlled. Polarized light and Nomarski optics detected the presence of a refractile boundary, such as that caused by the surface of cells covered with membrane. This refractile boundary is different from the optical artifact called edge birefringence. Dyes that bind to hydrophobic sites have been applied to the preparations to identify the membrane surface. Fluorescence occurred with and without the refractile boundary, but there were quantitative differences in the time of fluorescence and the intensity. Electrical measurements gave no evidence of the presence of membranes similar to those occurring in intact giant axons and other cells. A refractile cytoplasmic containing boundary can be selectively induced at the interface of cytoplasm and "membrane solutions". At the present time, there is only light optical evidence for its presence. This new surface appears to be the product of the coalescence of large vesicular inclusions that form on the application of test solutions. Ultrastructural and electrical data will be required to determine if this surface is a membrane and, if it is, whether it is functional.

Annual Report

July 1, 1973 through June 30, 1974

Laboratory of Experimental Neurology, Intramural Research
National Institute of Neurological Diseases and Stroke

William F. Caveness, M. D.

General Statement

This is a small laboratory with five permanent and one temporary position. Its productivity is dependent upon the conceptual and technical competence of each of its staff, the effective contract support for processing of anatomical specimen and supply of newborn monkeys, and the collaboration of scientists at Harvard University, and the Armed Forces Institute of Pathology.

This activity is a continuation of a primate laboratory established at Columbia University in 1958 as an adjunct to the Seizure Clinic for Children, Columbia Presbyterian Medical Center, both under my direction. Its initial purpose was to better understand clinical seizure patterns in infants and young children by studying the evolving seizure patterns in the developing brain of the monkey. The first eight years of this investigation is summarized in Ontogeny of Focal Seizures, chapter 19, in Basic Mechanisms of the Epilepsies, Little Brown and Co., 1969.

In 1961, in collaboration with Brookhaven National Laboratories, a series of studies were begun on the delayed effects of x-irradiation on the cerebral cortex of the Macaca mulatta. This was prompted by the clinical observations on the wards of the Neurological Institute of New York of delayed spinal cord necrosis that followed in a manner of months the unintentional exposure of the cord during irradiation therapy for neoplasms of the nasopharynx, lung or mediastival structures. The result of the first six years of these investigations are set forth in Pathogenesis of X-irradiation Effects in the Monkey Cerebral Cortex, Brain Research, 1968, 7: Special Issue.

The Laboratory was established within the Intramural Program, NINDS, NIH, April 1969 with the physical transfer of records and equipment from New York to Bethesda in March 1970.

Current Scientific Effort:

1) Ontogeny of Focal Seizures in the Monkey.

Earlier electrographic and clinical findings, indicative of the dependence of the seizure pattern on regional organization in the central nervous system, led us to

postulate that while the cerebral cortex in the newborn is able to support focal paroxysmal activity, it is dependent upon the subcortical somatotopically linked nuclear masses and circuits for the full development of focal abnormality, its propagation to other parts of the cortex and to a common final pathway that results in the clinical seizure. In the 24 month old (pubescent) monkey, on the other hand, the propagation is under the control of cortical circuitry.

To test this concept, two experimental procedures were carried out just prior to penicillin induced focal activity in the cortical motor face area. By undercutting the site of activation, the propagation was blocked in the newborn and unimpeded at 24 months of age. Interrupting the intracortical connections by circumsecting the activation site had negligible effect in the newborn, but blocked the propagation at 24 months. These findings support the concept that the effective neuronal organization for the propagation of focal paroxysmal activity shifts from subcortical to cortical with maturation. The anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations. At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits are well demarkated. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Regional Blood Flow Studies: A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us, it seemed wise to determine the natural course of events by noting the regional increase in blood flow, using the ^{14}C-labeled antipyrine method developed by Sokoloff et al, at sequential intervals after the induction of the seizure. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe. The long range goal is to identify essential neuronal systems that may be chemically coded.

A preliminary survey, with seventeen animals has provided the experience in standardizing the method and demonstrating its feasability in delineating patterns of increased flow in cortical and subcortical structures with the advance of electroencephalographic and clinical seizure patterns. For better anatomical localization the sections for autoradiography have been obtained in coronal, sagittal and longitudinal planes, respectively, in three series of newborn monkeys. We are just now prepared to systematically search

for sequential changes in seizure propagation with this method.

Concomitant with the preceding we have studied nine fully adult, 10 Kilo, monkeys for changes in regional blood flow that accompany the spontaneous cessation of seizures. We have found a quite different pattern in these when contrasted with those whose blood flow was determined during the height of a seizure. For example, Monkey # C-1, at the cessation of a Jacksonian seizure: The Motor Cortex showed a circumscribed blood flow increase, bilaterally, with the greatest extent on the right, the side of pencillin injection. The Caudate showed bilateral increase. The Globus Pallidus, medial aspect, was bilaterally increased with the greatest degree on the right. The Thalamus had no increase in VL, but a marked increase in VPL and VPm with the greatest extent on the right. The Cerebellum had a pronounced difference between the two sides with the left anterior and posterior lobes showing 40% and 35% greater blood flow than the homologous areas on the right. Monkey #C-2, during a Jacksonian seizure that had progressed to a generalized expression: The blood flow pattern showed far less laterality than that in #C-1, the Putamen and the Substantia Nigra being the only structures that showed a significant increase of the right over the left. When the actual blood flow determinations were compared with those of #C-1, the bilateral increase was found to be in the Premotor Cortex 85%, Sensory Cortex 40%, the Thalamic Nuclei VL 48%, CM 46%, with a comparative decrease in VPL of 27%. There was a very substantial bilateral increase in Pontine Nuclei, lateral 71% and medial 43%, the Cerebellar Cortex, anterior 104% and posterior 66%, and the Cerebellar Nuclei, Dentate 53%, Interpositus 34% and Fastigial 31%.

Significance: The results from these investigations should improve the understanding and management of the propagation of seizures in humans.

2) Delayed Effects of X-irradiation on the CNS of the Monkey.

The established model utilizes a single exposure of 3,500 rads to the right visual cortex in the young adult Macaca mulatta. This permits a precise measurement of functional changes as expressed in the visual evoked response. Sequential structural studies have shown ultrastructure evidence for cellular changes in carbohydrate and protein metabolism within days of exposure, alteration in the dendritic plexus within four to twelve weeks, and the proliferative and degenerative changes of radionecrosis in the exposed area four to five months following irradiction. Long-term functional alterations have included, first unilateral and then bilateral depression of evoked response and behavioral blindness. Long-term structural changes have included periodic brain swelling and retrograde transsynaptic degeneration within the visual system.

Three series of animals have been employed in the past two years to determine the mechanisms operating in the long-term phenomena. The first consisted of twelve monkeys with implanted cerebral spinal fluid catheter-reservoir systems, permitting sequential monitoring of the CSF pressure following irradiation. In eight animals there was relatively abrupt brain swelling, 18 to 35 weeks after irradiation, reflected by an increase in CSF

pressure, 3-4 times baseline values, with absence of visual evoked response and behavioral blindness in four, and marked VER depression in four. Three had papilledema. Seven of these, sacrificed between 20 and 36 weeks, one hour after i.v. Evans Blue, 2cc/kilo, showed proliferative and degenerative charges in the right occiput, deeply stained with dye. The swelling extended, beyond the irradiated and stained area, throughout the right hemisphere with distortion in brain structures. One sacrificed at 52 weeks, after resolution of the increased pressure and return of VER, had a fibrotic, non stained occipital lesion and no brain swelling. The remaining four animals showed variations in CSF pressure, seldom exceeding twice baseline value, over 52 weeks; moderate reduction in the visual evoked response; and moderately swollen brains, with Evans Blue staining of the irradiation site, at sacrifice.

The significant feature in the delayed brain swelling was the breakdown of the blood brain barrier that accompanied the delayed radiation lesion, with loss of serum fluid into the brain parenchyma. The impaired function was related to local destruction in the right visual cortex and widespread ipsilateral vasogenic edema and contralateral compression of the right and left visual pathways, respectively.

A second series of 18 monkeys was prepared with a subarachnoid catheter-reservoir system and irradiated in August, 1972. To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema the regional blood flow is being determined at five discrete points on the CSF pressure curve: 1) baseline, 2) ascending, 3) peak, 4) descending, 5) complete resolution, utilizing the C^{14} antipyrine technique.

A question of considerable interest is: What, if any, of these delayed effects would be observed if the exposure were divided and delivered to the whole brain in a manner similar to that now utilized by the Brain Tumor Study Group, of the Cancer Institute, in their controlled studies of human subjects. Their protocol calls for 6000 rads to be delivered to the whole brain over a period of six weeks, 200 rads mid plane dose per day, five days per week, with megavoltage equipment. To approximate this exposure in an animal model, we have irradiated the third series of monkeys with the Linear accelerator equipment at the Armed Forces Radiobiological Research Institute, that delivered 6000 rads to the whole brain, over a six week period, at 200 rads per day, with minor variations, five days a week, in four monkeys. To bracket the effect, we irradiated four animals with 4000 rads over four weeks, and four animals with 8000 rads over eight weeks. We are monitoring this series with clinical examinations, visual evoked responses, and fundascopic photography. One animal from each of the exposure doses plus a control will be sacrificed at 6, 12, 18, and 24 months respectively, if survival permits. To date, two of those with 6000 rads have shown papilledema from 8 and 12 weeks postirradiation, respectively. All four of those with 8000 rads have shown ataxia and dysmetria from 24 to 26 weeks postirradiation. Three have lost weight and one has abnormally gained weight.

Finally, the sequence of structural changes, proceding from intracellular to local

tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys and four 100 day old monkey fetuses have received cerebral injections of material from lesions in monkeys with postirradiation necrosis. Three monkeys have been immunosuppressed to determine whether this will accelerate or decelerate the appearance and extent of the lesions. Electronmicroscopic evaluation of the irradiated brain and cervical lymph nodes in nine monkeys have not revealed recognizable viral particles but have shown consistantly tubulo-reticular structures in endothelial cells, that have not been found in three controls. We believe these represent a cellular reaction to a virus or to an immune antigen.

Significance: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in therapeutic efforts, the use of nuclear energy, and future space exploration.

3) Vietnam Registry.

A registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the U.S. Navy and was implemented with the cooperation of the Surgeons General of the U.S. Army and U. S. Air Force. The purpose was to insure uniformity of data collection and to identify cases for present and future studies. The yield from 1967 to 1970 was 2,043 entries that included 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 22.2 years. Of the head injuries, 90% were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without evidence of alteration in consciousness. A formal report of the Registry content and an estimate of its usefulness has been transmitted to the Surgeons General, copy to the Director, Intramural Research.

Significance: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma.

Serial No. NDS(1)-69 LEN/OC 1693
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Functional and Structural Alterations Following X-irradiation of the Cerebral Cortex in the Monkey

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Hiroyuki Nakagaki, M.D.
Thomas L. Kemper, M.D.
Lorenz E. Zimmerman, M.D.
Mark O.M. Tso, M.D.

Cooperating Units: Harvard University Medical School
Armed Forces Institute of Pathology

Man Years

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description

Objectives: To determine the pathogenesis of early and late effects of irradiation on the central nervous system.

Methods Employed: The right visual cortex, in the young adult macaca mulatta, is exposed to 3,500 rads of x-irradiation in a single dose. Functional changes are monitored by evoked electroencephalographic responses from photic stimulation of the retina prior to and at biweekly intervals, following irradiation. The stimuli are programmed to provide single light flashes that may be averaged and a series of frequencies that range from three to thirty flashes per second in ten second epochs. The evoked response from the irradiated and non-irradiated visual cortex is led through a two-channel Grey-Walter, low frequency analyzer that provides histograms for the abundance of activity, or amplitude, for ten seconds at each of the frequencies employed. Thus one can compare directly the

amplitude of the evoked response from the irradiated cortex with that from the homologous non-irradiated cortex. In addition, the frequency analyzer detects delta activity, one to three cycles per second, when it appears as a reaction to injury. The averaged evoked response of a series of thirty-two flashes at one second intervals has provided a sensitive indicator of alteration in wave form that is especially useful when the differences between the irradiated and non-irradiated side are less pronounced.

Sequential anatomical examinations have been carried out by sacrificing three animals at 72 hours, 1, 4, 12, 20, 28, 44 and 144 weeks for routine, Golgi-Cox and electron microscopic preparations. Six animals have served as controls.

Major Findings: In the first ten months the functional changes included an initial and secondary unilateral depression in the photic evoked response in the right visual cortex. There was ultrastructure evidence for changes in carbohydrate and protein metabolism within three days of exposure, alteration in the dendritic plexus within 4 to 12 weeks, and radionecrosis, in the exposed area, within 4 to 5 months following irradiation. (Special Issue of Brain Res. 7: 1-120, 1968) In nine additional preparations, long-term functional alterations, up to 36 months, have included bilateral depression of evoked response and behavioral blindness. The long-term structural changes have included pericdic brain swelling, and retrograde transsynaptic degeneration within the visual system.

During the preceding fiscal year, three new experimental procedures have been exploited to define the mechanisms operating in the long-term phenomena.

The first consisted of twelve monkeys with implanted cerebral spinal fluid catheter-reservoir systems, permitting sequential monitoring of the CSF pressure following irradiation. In eight animals there was relatively abrupt brain swelling, 18 to 35 weeks after irradiation, reflected by an increase in CSF pressure, 3-4 times baseline values, with absence of visual evoked response and behavioral blindness in four, and marked VER depression in four. Three had papilledema. Seven of these, sacrificed between 20 and 36 weeks, one hour after i.v. Evans Blue, 2 cc/kilo, showed proliferative and degenerative changes in the right occiput, deeply stained with dye. The swelling extended, beyond the irradiated and stained area, throughout the right hemisphere with distortion in brain structures. One sacrificed at 52 weeks, after resolution of the increased pressure and return of VER, had a fibrotic, non stained occipital lesion and no brain swelling. The remaining four animals showed variations in CSF pressure, seldom exceeding twice baseline value, over 52 weeks; moderate reduction in the visual evoked response; and moderately swollen brains, with Evans Blue staining of the irradiation site, at sacrifice.

The significant feature in the delayed brain swelling was the breakdown of the blood brain barrier that accompanied the delayed radiation lesion, with loss of serum fluid into the brain parenchyma. The impaired function was related to local destruction in the right visual cortex and widespread ipsilateral vasogenic edema and contralateral

compression of the right and left visual pathways, respectively.

A second series of 18 monkeys was prepared with a subarachnoid catheter-reservoir system and irradiated in August, 1972. To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema the regional blood flow is being determined at five discrete points on the CSF pressure curve: 1) base-line, 2) ascending, 3) peak, 4) descending, 5) complete resolution, utilizing the C^{14} antipyrine technique.

A question of considerable interest is: What, if any, of these delayed effects would be observed if the exposure were divided and delivered to the whole head in a manner similar to that now utilized by the Brain Tumor Study Group, of the Cancer Institute, in their controlled studies of human subjects with brain tumor. Their protocol calls for 6000 rads to be delivered to the whole brain over a period of six weeks, 200 rads mid plane dose per day, five days per week, with megavoltage equipment. To approximate this exposure in an animal model, we have irradiated the third series of monkeys with the Linear accelerator equipment at the Armed Forces Radiobiological Research Institute, that delivered 6000 rads to the whole brain, over a six week period, at 200 rads per day, with minor variations, five days a week, in four monkeys. To bracket the effect, we irradiated four animals with 4000 rads over four weeks, and four animals with 8000 rads over eight weeks. We are monitoring this series with clinical examinations, visual evoked responses, from the whole head and with fundascopic photography. One animal from each of the exposure doses plus a control will be sacrificed at 6, 12, 18, and 24 months respectively, if survival permits. To date, two of those with 6000 rads have shown papilledma from 8 and 12 weeks postirradiation, respectively. All four of those with 8000 rads have shown ataxia and dysmetria from 24 to 26 weeks postirradiation. Three have lost weight and one has abnormally gained weight.

Finally, the sequence of structural changes, proceeding from intracellular to local tissue, to remote and generalized effects, with remissions and exacerbations, strongly suggest isoimmune reactions and/or the activation of an infectious agent (slow and latent viruses). To test whether such a factor is present and transmissible, two newborn monkeys and four 100 day old monkey fetuses have received cerebral injections of material from the lesions in monkeys with postirradiation necrosis. Three monkeys have been immuno-suppressed to determine whether this will accelerate or decelerate the appearance and extent of the lesions. Electronmicroscopic evaluation of the irradiated brain and cervical lymph nodes in nine monkeys have not revealed recognizable viral particles but have shown consistantly tubuloreticular structures in endothelial cells, that have not been found in three controls. We believe these represent a cellular reaction to a virus or to an immune antigen.

Significance: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in

therapeutic efforts, the use of nuclear energy, and future space exploration.

Proposed Course: Completion of the studies on alteration in regional cerebral blood flow in relation to the postirradiation vasogenic edema; continuation of the monitoring of functional and structural changes that follow whole brain irradiation in regimens that simulate human therapeutic trials; and the continuation of the search for the basic mechanisms in the delayed breakdown in neural tissue following exposure to ionizing irradiation.

Honors and Awards: None

Publications:

Caveness, W. F., Tanaka, A., Hess, K.H., Kemper, T. L., Tso, M.O.M., and Zimmerman, L. E.: Delayed brain swelling and functional derangement after x-irradiation of the right visual cortex in the macaca mulatta. Radiat. Res. 57, 104-120, 1974.

Serial No. NDS(I)-69 LEN/OC 1694
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ontogeny of Focal Seizures

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Hideo Ueno, M. D.
Yoriaka Yamashita, M. D.
Thomas L. Kemper, M. D.

Cooperating Units: Harvard University Medical School

Man Years:

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

Objectives: To better understand the evolving seizure pattern in the developing brain.

Methods Employed: Focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution into the hand-face area of the motor cortex of the Macaca mulatta at successive age levels from birth to young adulthood.

The onset and elaboration of the attack pattern is simultaneously recorded by electrocorticography, electromyography, and clinical observation.

The cerebra of the experimental, and a control, series of animals are examined for the level of development in cells, neuropil and myelin with attention to the motor cortex and subcortical tracts that are deemed relevant.

Major Findings: A significant feature of this investigation, in the Macaca mulatta, has been the apparent dependency of the immature motor cortex on thalamic and other subcortical structures in the development and propagation of the paroxysmal discharge.

To test this concept, the cortical-subcortical pathways beneath the face area in the precentral gyrus were sectioned in eight newborn monkeys. The undercut cortex was then injected with 25,000 units of penicillin. The paroxysmal activity recorded from an array of cortical electrodes developed within the undercut area and, except for limited and transient expression, did not spread beyond this region, suggesting a marked dependency upon the subcortical circuitry for propagation of the focal abnormality. Another procedure, in eight newborn animals, involved a supial section of the cortex on four sides that separated the face area from the remainder of the precentral gyrus and the frontal cortex anterior to the arcuate sulcus. When penicillin was injected into the circumsected cortex the paroxysmal activity developed and spread beyond the cortical sections in the superior and inferior motor strip and to other regions of the cortex with concomitant clinical expression of the seizure, suggesting a limited dependence upon the continuity of the cortex for propagation of the abnormal activity.

Both procedures were carried out in four monkeys at 24 months of age. The results, in contrast to the findings in the newborn, indicate far greater dependence on the cortex for the development and propagation of the paroxysmal activity.

During the past year, the anatomical substrate for the seizure activity in the cortex has been extensively investigated with particular attention to the developing fiber systems, as delineated by Nauta techniques, and the developing pyramidal and stellate cells as demonstrated in Golgi preparations.

At birth, cortico-cortical connections are poorly developed, and cortico-subcortical circuits provide the major pathway for the propagation of cortical activity. In contrast, cortico-cortical connections are prominent at 24 months of age. This increase in cortical connectivity is reflected in the postnatal architectonic development of layers II and III, the loci of the most dramatic events of the postnatal development.

Significance to Bio-Medical Research and the Program of the Institute: The results from this investigation should improve the understanding and management of the age dependent seizure patterns in humans.

Proposed Course: A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. For us, it seemed wise to determine the natural course of events by

noting the regional increase in blood flow, using the ^{14}C-labeled antipyrine method developed by Sokoloff et al, at sequential intervals after the induction of the seizure. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe. The long range goal is to identify essential neuronal systems that may be chemically coded. See Project Number 1897.

Honors and Awards: None

Publications:

Caveness, W.F., Echlin, F.A., Kemper, T. L., and Kato, M.: The propagation of focal paroxysmal activity in the Macaca mulatta at birth and at 24 months. Brain 96: 757-764, 1973.

Kemper, T.L., Caveness, W. F., and Yakovlev, P.I.: The neuronographic and metric study of the dendritic arbours of neurons in the motor cortex of Macaca mulatta at birth and at 24 months of age. Brain 96: 765-782, 1973.

Serial No. NDS(I)-71 LEN/OC 1897
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regional Blood Flow Studies

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Hideo Ueno, M. D.
Yoriaka Yamashita, M. D.
Louis Sokoloff, M. D.

Cooperating Units: Laboratory of Cerebral Metabolism, NIMH

Man Years:

Total:	2.0
Professional:	.8
Other:	1.2

Project Description

See 1693. To investigate the alteration in cerebral blood flow that accompanies the evolution and resolution of vasogenic edema that follows exposure to the cerebral cortex to ionizing irradiation, a series of 18 monkeys was prepared with a spinal subarachnoid catheter-reservoir system and irradiated in August 1972. Utilizing the ^{14}C antipyrine technique of Sokoloff, Reivich and others, the regional blood flow is being determined at five discrete points on the CSF pressure curve; 1) baseline, 2) ascending, 3) peak, 4) descending, 5) complete resolution. A correlation of regional blood flow with the structural changes in the brain at each period will be established.

See 1694. A logical extension of the investigation of the propagation of focal seizures in the newborn monkey is a search for the subcortical circuits and nuclear masses that are involved in the seizure activity. Previous studies by others have attempted to define "preferential" pathways by depth recording or by selective destruction of subcortical structures. In lieu of duplicating their efforts, which are limited by the position of the probes, it seemed wise to determine the natural course of events by the use of

blood flow techniques, using the ^{14}C antipyrine method at sequential intervals after the induction of the seizure. Two advantages in exploring subcortical mechanisms in the newborn will be exploited. First, the level of development and integration of the cortex is such that the better developed subcortical systems are likely to have clearer expression. Secondly, the propagation of seizure activity, at this age, is protracted in time providing a better opportunity to observe the sequential involvement of neuronal aggregates. Once the natural progression is delineated, then one may appropriately investigate individual components with depth recording or interruption of activity by a cryoprobe.

Major Findings to Date:

A preliminary survey, with seventeen animals has provided the experience in standardizing the method and demonstrating its feasability in delineating patterns of increased flow in cortical and subcortical structures with the advance of electroencephalographic and clinical seizure patterns. For better anatomical localization the sections for autoradiography have been obtained in coronal, sagittal and longitudinal planes, respectively, in three series of newborn monkeys. We are just now prepared to systematically search for sequential changes in seizure propagation with this method.

Concomitant with the preceding we have studied nine fully adult, 10 Kilo, monkeys for changes in regional blood flow that accompany the spontaneous cessation of seizures. We have found a quite different pattern in these when contrasted with those whose blood flow was determined during the height of a seizure. For example, Monkey #C-1, at the cessation of a Jacksonian seizure: The Motor Cortex showed a circumscribed blood flow increase, bilaterally, with the greatest extent on the right, the side of penicillin injection. The Caudate showed bilateral increase. The Globus Pallidus, medial aspect, was bilaterally increased with the greatest degree on the right. The Thalamus had no increase in VL, but a marked increase in VPL and VPm with the greatest extent on the right. The Cerebellum had a pronounced difference between the two sides with the left anterior and posterior lobes showing 40% and 35% greater blood flow than the homologous areas on the right. Monkey #C-2, during a Jacksonian seizure that had progressed to a generalized expression: The blood flow pattern showed far less laterality than that in #C-1, the Putamen and the Substantia Nigra being the only structures that showed a significant increase of the right over the left. When the actual blood flow determinations were compared with those of #C-1, the bilateral increase was found to be in the Premotor Cortex 85%, Sensory Cortex 40%, the Thalamic Nuclei VL 48%, CM 46%, with a comparative decrease in VPL of 27%. There was a very substantial bilateral increase in Pontine Nuclei, lateral 71% and medial 43%, the Cerebellar Cortex, anterior 104% and posterior 66%, and the Cerebellar Nuclei, Dentate 53%, Interpositus 34% and Fastigial 31%.

Significance to Bio-Medical Research and the Program of the Institute: The investigation of regional blood flow alteration in brain edema following irradiation should add to our understanding of the effects of brain swelling, from whatever cause.

The global appraisal of the propagation of focal seizure activity by the regional cerebral blood flow method should provide a clearer understanding of the role of subcortical structures.

Proposed Course: Completion of the study of cerebral edema. Continuation of the study of seizure propagation in the newborn.

Honors and Awards: None

Publications:

Kato, M., Ueno, H., and Black, P.: Regional cerebral blood flow of the main visual pathways during photic stimulation of the retina in intact and split-brain monkeys. Exp. Neurol. 42, 65-77, 1974.

Serial No. NDS(I)-72 LEN/OC 1952
1. Laboratory of Experimental Neurology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Vietnam Registry, Analysis of Yield

Previous Serial Number: Same

Principal Investigator: William F. Caveness, M. D.

Other Investigators: Berkley L. Rish, CAPT (MC) USNR

Cooperating Units: Office of the Surgeon General, USN
Office of the Surgeon General, USA

Man Years:

Total:	1.0
Professional:	.5
Other:	.5

Project Description

Objectives: To insure uniformity of data collection in combat head injuries and to identify cases for present and future studies.

Methods Employed: A Registry for Head and Spinal Cord Injuries, as they occurred in military combat in Vietnam, was developed at the request of the Surgeon General of the Navy and was implemented with the cooperation of the Surgeons General of the Army and Air Force. The yield from field surgeons, 1967-1970, was 2,043 entries.

Major Findings: The Registry includes 1,683 head injuries, 329 spinal cord injuries and 31 combinations of the two. The average age at time of injury was 22.2 years. Of the head injuries, 90% were the result of explosively propelled missiles, 10% the result of vehicular accidents, falls, or blunt objects. In 85% there was local brain destruction, as evidenced by penetration of the dura with cortical laceration. 60% of these were accompanied by immediate loss in consciousness, by history. 30% were in coma, responding only to pain at time of examination, that was generally within six

hours of injury. This provides an opportunity to group selected cases by regions of focal damage, with and without evidence of alteration in consciousness.

Significance to Bio-Medical Research and the Program of the Institute: The observations during the acute phase of injury in these cases are more uniform and probably more accurate than any previous series of comparable size. This provides an extraordinary opportunity for studies of prognostic factors and the natural history of disabilities in central nervous system trauma.

Proposed Course: The evaluation of these data is being continued with particular attention to wound characteristics and clinical events during the acute phase of injury.

Honors and Awards: None

Publications:

Caveness, W. F., and Rish, B.L.: Structural and functional derangement in head injuries from Vietnam. An exhibit presented at the following meetings: American Association of Neurological Surgeons, April, 1972, American Neurological Association, June, 1972, Congress of Neurological Surgeons, October, 1972, Association of Military Surgeons of the United States, December, 1972, and American Medical Association, June, 1974.

Rish, B. L., and Caveness, W. F.: Relation of prophylactic medication to the occurrence of early seizures following craniocerbral trauma. J. Neurosurg. 38: 155-158, 1973.

Serial No. NDS(I)-62 LB/OC 935
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2087.

Serial No. NDS(I)-62 LB/OC 936
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2091.

Serial No. NDS(I)-62 LB/OC 939
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2088.

Serial No. NDS(I)-62 LB/OC 940
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ionic Permeabilities of Excitable Membranes: Effects of Chemical Agents

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2088.

Serial No. NDS(I)-65 LB/OC 1240
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Molecular Biophysics - Physical Properties of Membranes and Simple Membrane-Like Systems

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2090.

Serial No. NDS(I)-72 LB/OC 1947
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cytoplasmic Water Structure

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2092.

Serial No. NDS(I)-72 LB/OC 1948
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrical Properties of Small Patches of Excitable Membrane

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2089.

Serial No. NDS(I)-72 LB/OC 1950
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Rapid Analysis of Excitable Membranes by Means of Computer Controlled Voltage Clamp

Previous Serial Number: Same

Principal Investigators: W. J. Adelman, Jr., Ph.D., L. Binstock, B.S., M.S.

Other Investigators: B. Cox, Ph.D., J. Shaw, B.S., M.A., F. Sachs, Ph.D., H. Walters

Cooperating Units: None

Man Years:

Total:	3.4
Professional:	1.9
Others:	1.5

Project Description:

Objectives: The Laboratory of Biophysics has as one of its research goals an analysis of the membrane events producing the nerve impulse. The propagated nerve impulse is an explosive event during which neither the current nor the voltage (action potential) is under the control of the experimenter. The voltage clamp employs electronic feedback to set the voltage across the membrane at a time and space controlled value in order to observe membrane conductances directly as time-varying currents. This approach requires numerous records to be obtained over a reasonably short period of time with an over-all band pass from DC to hundreds of thousands of Hz. The object of this project has been to develop, design, construct, and program an on-line data acquisition system capable of performing the variety of voltage clamp experiments required by the Laboratory's experimental programs. In addition, this data acquisition system has been designed to provide data output in a form compatible with large digital computer data reduction and analysis.

Methods Employed: Two systems have been designed and constructed. The first system used by the Laboratory consists of a small 8-bit mini-computer with teletyper, program control, pulse making electronics and 9-track digital magnetic tape. This system has proven the concept of automating the voltage clamp processes as well as acquiring data in a form that can be immediately analyzed by large computers. During this fiscal year this system has been

updated with respect to improving both hardware and software. The data acquisition phase of the work done by this data acquisition system is done at the Marine Biological Laboratory in Woods Hole, Mass., during the spring and summer. The data tapes produced by this system are processed by the Laboratory of Biophysics' PDP-11 mini-computer, using techniques and programs developed for this purpose during the fall and winter. The results of this phase are tables of membrane conductances, and other parameter values as a function of time and voltage, which are in a condensed form suitable for use by DCRT's large computing facility, which is used for all curve-fitting operations.

The second system was designed primarily for control of and data acquisition from experiments performed primarily in Building 36, in Bethesda, Md. This system is capable of acquiring data of higher rates up to 10^6 power sample per second while producing a wider variety of precision control pulses. As this system has a larger core memory and a disk, more sophisticated programs for experimental control can be and have been written, in addition a more versatile magnetic tape recording device incorporated in the system, data acquisition and storage can be made more efficient. A CRO graphics terminal allows greater interaction between the experimentalist and the data acquisition system, allowing considerable feedback between the experimenter and the experiment. This system has also been programmed for fast Fourier analysis of fluctuating responses in voltage clamped membranes and is capable of generating power spectrum of these fluctuations. Details of this application are provided in the report No. NDS(I)-74 LB/OC 2089.

Major Findings: Work this year has been devoted to the development of new computer programs and analysis techniques for the most efficient use of data acquisition system of the first data system used in Woods Hole, Mass. This phase of the project is now virtually complete.

Analysis of data collected with this system and during the summer of 1973 is now underway, making use of the powerful digital computers in DCRT. The analysis of the data obtained by the smaller data acquisition system is processed initially using a Sigma-7 digital computer in Woods Hole, Mass., and the Laboratory's larger data acquisition system in Bethesda. Final data analysis is performed on the PDP-10 computer in DCRT. This enables us to make use of powerful, curve-fitting, and modeling programs such as MLAB.

Software has now been developed to use the second data acquisition system to computer control the voltage clamp for Myxicola axon. Data is readily obtained and stored on magnetic tape. The program and analysis used is the same as that used for squid axon with some possible modifications for Myxicola axon. When more experiments and analysis are added to the present data, the first full description of Myxicola axon conductances will have been obtained by a computer controlled voltage clamp.

Significance to Biomedical Research and the Program of the Institute: The use of the computer program controlled voltage clamp enables one to quickly collect data and do many experiments in a short time.

The second data acquisition system in Bethesda, Md., has now been interfaced to three separate laboratories performing rather different experiments. Many difficult experiments that have not been possible before can now be done in greater detail. The turnover time from experiment to final analysis by conventional means has been very time consuming. The output rate of information gathering and processing by the new methods has been increased many fold and information on the structure and mechanism of the membrane can thus be obtained more accurately and much sooner than by any other method.

Proposed Course of the Project: The computer program controlled voltage clamps will continue to be used in much greater detail in the studies of various membranes both here and at Woods Hole, Mass. We expect that most voltage clamp experiments in the future will use the computer controlled technique. It is expected that software development for these data acquisition systems will continue so as to make them available in more experimental situations and in answering more sophisticated questions. Essentially all software is in FORTRAN, and is therefore readily exportable to other laboratories, institutes and universities. Therefore, a publication is planned to describe and document the hardware designs and software programs unique to these data acquisition systems.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-72 LB/OC 1966
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Function and Structure of Membrane Ionic Channels Obtained from Studies Involving Ion Interactions with the Membranes of Giant Axons

During FY 1974 this project was incorporated with Serial No. NDS(I)-74 LB/OC 2087.

Serial No. NDS(I)-72 LB/OC 1967
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrical Fluctuations of Excitable Membranes

Previous Serial Number: Same

Principal Investigator: K. S. Cole, Ph.D.

Other Investigators: H. M. Fishman, Ph.D. and T. L. Hill, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

State University of New York at Albany, Albany, New York

Laboratory of Molecular Biology, NIAMD, NIH

University of California, Berkeley, California.

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description: Project terminated.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LB/OC 2087
1. Biophysics
2. Office of the Chief
3. Pethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Function and Structure of Ionic Channels: Ion Gating Mechanisms

Previous Serial Number: None and incorporating Serial No. NDS(I)-62 LB/OC 935 and NDS(I)-72 LB/OC 1966

Principal Investigators: W. J. Adelman, Jr., Ph.D. and R. E Taylor, Ph.D.

Other Investigators: Brad Cox, Ph.D., Joseph P. Senft, Ph.D., Y. Palti Ph.D., E. Rojas, Ph.D., C. M. Armstrong, Ph.D., M.D., F. Bezanilla, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

Laboratory of the Marine Biological Association, Plymouth, England

Laboratorio de Fisiologia Cellular, Montemar, Chile

Juniata College, Huntingdon, Pa.

The Technion, School of Medicine, Haifa, Israel

Man Years:

Total:	1.8
Professional:	1.8
Other:	0.0

Project Description:

Objectives:

a. Monovalent cation conductances: To determine the behavior of excitable membrane channels as a function of ionic species, concentrations, and interactions. To relate this behavior to ionic channel structures as a basis for the nerve impulse. To develop a quantitative model capable of describing the phenomenological behavior of the axon so as to account for its physiological function. To investigate the role of ion accumulation in periaxonal spaces in modifying ionic channel conductances. By consideration of the measured kinetics of the channel processes as a function of voltage and ionic concentrations, to test certain classes of microscopic

channel models which have been proposed.

b. Divalent cation conductances: To study the voltage dependent permeabilities of the nerve membrane to divalent ions. To determine the kinetics of divalent ion flow through both tetrodotoxin (TTX) sensitive and TTX insensitive channels. To test various hypotheses for the action of divalent ions in nerve membrane function.

Methods Employed:

a. Monovalent cation conductances: Giant axons from the squid Loligo pealei are current-clamped in sea water solutions modified to give repetitive firing to long duration constant currents. These axon are precision voltage clamped in the same solutions using the data acquisition system described in Project Report No. NDS(I)-72 LB/OC 1950. Analytical programs have been developed to determine ionic channel kinetics through the measurement of appropriate parameter values from voltage clamp data such that predicitions can be made relevant to the repetitive responses and their adaptation obtained upon current clamping these axons. Experimental and analytical programs have been devised to consider the role of periaxonal potassium accumulation in modifying both the activity and measurement of ionic channel conductances.

b. Divalent cation conductances: Giant axons of the squid Dosidicus gigas, Loligo forbesii and Loligo vulgaris are internally perfused with Kf + Tris or KF + Tris + sucrose and bathed in artificial sea water containing ^{45}Ca, ^{28}Mg. or mixtures of ^{45}Ca-^{22}Na or ^{45}Ca-^{28}Mg. Resting influxes and extra influxes during voltage clamp pulses are measured by collecting and counting the perfusate. Calcium currents are measured in internally perfused giant axons of the squid Loligo pealei by the voltage clamp method.

Major Findings:

a. Monovalent cation conductances: Extensive data was collected in the summer of 1973 to provide the means by which the analytical programs could be tested, revised, and improved. Most of the fall and winter has been occupied in developing the software for these analytical programs and for performing preliminary analyses on this data. This phase of the project is now virtually complete. As described below, in the Proposed Course of the Project, much work remains before a new quantitative model for squid axon behavior can be verified. The analysis of the role of the periaxonal space has been completed and published. This work was presented at an invited lecture in the Colloquium on Membranes, Ions and Impulses at the 1974 Federation Meeting. An extension of this work to the possible role of glial cells in epileptogenesis was presented at the Eastern Electroencephelography Meeting this past winter. A number of models have been developed and programs have been written for testing these models.

b. Divalent cation conductances: Using depolarizing pulses of various durations the extra calcium influx occurs in two phases, neither of which is

affected by internal tetraylethylammonium (TEA) but the early phase is eliminated by external TTX. The results have been analysed and are consistent with the conclusion that the TTX sensitive component is flowing through the sodium channels. If so, P_{Na}/P_{Ca} is estimated to be 188 ± 80. However, measurements using ^{22}Na and ^{45}Ca simultaneously indicate that the time courses of the TTX sensitive calcium and sodium entry may not be identical. It was confirmed for Loligo, as had been previously shown for Dosidicus that the magnitude and time course of the sodium entry during a depolarizing pulse deduced from electrical measurements is the same as that measured with ^{22}Na. Using ^{28}Mg or mixtures of ^{28}Mg and ^{45}Ca a TTX insensitive component for Mg influx was found. The time course of the Mg influx differed from that of Ca and both differed from that for K. The magnesium influx was considerably larger than the TTX insensitive calcium flux. In fact, this flux was so large that if it represented a current through a channel it should have been seen in the experiments of Meves and Vogel. Further work is required to characterize these influxes. This work has been submitted to the Journal of Physiology for publication.

A significant, although preliminary, finding was observed showing that a small current carried by calcium ions in perfused and voltage clamped squid axons. This current had a time course during a voltage clamp pulse very similar to that of sodium. This does not confirm the observations reported by Meves and Vogel in 1973 who saw only a slow, TTX sensitive calcium current component. The difference may be due to the fact that they used a low internal ionic strength (25 mM CsF) perfusion solution while we used 290 mM CsF. On the basis of some rather doubtful assumptions which need to be checked we got a P_{Na}/P_{Ca} ratio of about 66. These results were reported at the Federation Meeting in Atlantic City, N. J.

Significance to Biomedical Research and the Program of the Institute:

a. Monovalent cation conductances: Successful termination of this aspect of the project should provide us with the means for predicting a wide variety of neurophysiological behavior related directly to the molecular nature of individual nerve membrane ionic channels.

b. Divalent cation conductances: The knowledge of movement of divalent ions through sodium channels and the interference of calcium ions with sodium currents are of great value in our attempt to define the structure of the sodium channel. The TTX insensitive calcium and magnesium voltage dependent influx is possibly carried by a mechanism distinct from the classical and sodium potassium channels, and may be directly related to the presumed calcium entry at nerve terminal responsible for the release of transmitter substances.

Proposed Course of the Project:

a. Monovalent cation conductances: Data collection and analysis will continue, with emphasis on collecting data for quantitative modeling and performing critical experiments for choosing between possible membrane models. In particular interest will be the examination of the two-barrier single site

model described in Report No. NDS(I)-74 LB/OC 2091.

b. Divalent cation conductances: Experiments are planned with Dr. Armstrong of the University of Rochester to try to resolve the questions posed above with respect to calcium and magnesium action. There is still an outside chance that some calcium ions might be entering before sodium ions in an electroneutral exchange process.

Honors and Awards:

Dr. Adelman was invited to present a review of his work at the Membranes, Ions, and Impulses Colloquium at the Federation Meeting in Atlantic City, New Jersey; to prepare a review for Progress in Neurobiology; and to coedit a book on Biophysical Methods. Dr. Taylor was invited to prepare a review for the 1974 edition of Annual Reviews of Physical Chemistry.

Publications:

Adelman, W. J., Jr., Palti, Y. and Senft, J. P.: Potassium ion accumulation in a periaxonal space and its effect on the measurement of membrane potassium ion conductance. J. Membrane Biol. 13: 387-410, 1973.

Taylor, R. E: Excitable membranes. In Annual Reviews of Physical Chemistry. (In Press)

Serial No. NDS(I)-74 LB/OC 2088
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Function and Structure of Membrane Ionic Channels: Pharmacology and Ionic Selectivity

Previous Serial Numbers: None and incorporating Serial No. NDS(I)-62 LB/OC 939 and NDS(I)-62 LB/OC 940

Principal Investigators: L. Binstock, B.S., M.S., D. L. Gilbert, Ph.D.

Other Investigators: R. DiPolo, Ph.D., G. Ehrenstein, Ph.D., R. Henkin, Ph.D., R. J. Lipicky, Ph.D., R. Manalis, Ph.D. and I. Stillman, M.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

National Heart and Lung Institute, NIH

Division of Computer Research and Technology, NIH

Man Years:

Total:	2.8
Professional:	2.3
Other:	0.5

Project Description:

Objectives: To study the ionic current flow across the nerve and the post-synaptic membrane without the complication of excitation and propagation in terms of individual ion currents and to observe the effect of changes in normal internal and external environments. These environments are altered by addition of various chemical and pharmacological agents as well as by changing the ionic environment. The long range objectives are the interpretation of nerve and synapse function in terms of ion movements and the elucidations of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed on the following preparations: a. Myxicola giant axon, b. squid giant axon and c. squid giant synapse. Internal perfusion and injection are also employed on the Myxicola giant axon. For many experiments on the giant axon of Myxicola, the voltage clamp is under computer control. In addition, the computer provides both on and off line analysis of the data.

Major Findings: a. Myxicola giant axon: New measurements made on electron micrographs of the Schwann cell space of the Myxicola giant axon yield a resistance of 2.3 Ωcm^2.

Sea Nettle Toxin (SNTX), prepared in a different way somewhat similar to that used on the squid giant axon, produced no appreciable change in ionic currents at a concentration of 80 mg/liter.

Veratrine at a concentration of about $5x10^{-5}$M and veratridine at a concentration of about $1x10^{-6}$M produce a decrease in both Na and K currents with a possible change in τ_n slowing it down. The action potentials are not prolonged. More work is needed to see why there is no prolongation of the action potential or if the inactivation is changed.

Initial experiments with 100 mM cesium appears to block steady state inward potassium currents through the K-channel with an external concentration of 340 mMK. Without cesium the potassium appears to enter the Na channel when clamped at the normal sea water resting potential. Inward potassium currents are observed as well as a change in the apparent equilibrium potential. With external cesium inward and outward early currents through the Na channel are blocked.

Internal perfusion is becoming more reliable but still poses a problem. Action potentials are larger than they have been but usually disappear with a solution change. Perfusion rate is about 1 drop/15 seconds giving several changes of solution in less than a minute. It is necessary to large axons and to soften the gelled axoplasm. Various enzymes such as Pronase, 10 mg/100 ml; L-cysteine, 6 mg/100 ml; and Chymotrypsin, 2 mg/100 ml have been used to clear the axoplasm. Currents can be obtained very readily even with low resting potentials by holding at a reasonable potential.

b. Squid giant axons: A dose-response relationship of mercuric chloride on the ionic currents in the squid giant axon was determined. At a concentration of about 0.05 mM, the sodium currents are reduced by about 50 per cent. A poor recovery of the sodium currents after treatment with the mercuric ion could be obtained with 2-mercaptoethanol. Perhaps, the binding of the mercuric ions to the membrane sites is so strong that 2-mercaptoethanol cannot remove too much of the bound mercury. Another compound, N-ethylmaleimide, freshly prepared was also tried. This compound also inhibits sulfhydryl groups, but acts by an additional reaction instead of mercaptide formation. At a high concentration of 2 mM, it appeared to inhibit the sodium currents after about twenty minutes. However, no recovery was obtained with 1 mM 2-mercaptoethanol.

We have previously shown that diphenylhydantoin (DPH) inhibits the activated sodium conductance in the squid giant axon. A computer analysis of the Hodgkin-Huxley model of the space clamped squid giant axon revealed that the firing rate of the spontaneously active axon is not appreciably decreased by a decrease in the activated sodium conductance. In the model,

spontaneous activity was induced by an appropriate potential shift of the Hodgkin-Huxley "m" parameter. Such a shift, according to the surface charge hypothesis, could be obtained by decreasing the external divalent cation concentration. We decided to test the adequacy of our conclusion concerning the mechanism of DPH action by determining its effect on the firing rate of the space clamped spontaneously active squid giant axon. Induced spontaneous activity of the axon was obtained by raising the temperature to 12° C and decreasing the total divalent cation concentration to only 5 mM calcium. The spontaneous activity under these circumstances was about 50 Hz. Concentrations of 1.10^{-5} to 1.10^{-4}M DPH abolished the spontaneous activity and finally blocked the action potentials. The spontaneous and repetitive activity disappeared without a change in the intrinsic firing frequency. Thus, these experiments are consistent with the hypothesis that a decrease in the activated sodium conductance is the main effect of DPH.

c. Squid giant synapse: In an other study, experiments were performed on the squid giant synapse to determine if the post-synaptic current (PSC) is potential dependent. Preparations were bathed in an oxygen saturated artificial sea water buffered to a pH of 7.8 with Tris, usually at 10°C. The post-synaptic membrane potential was clamped at the resting potential and the PSC was recorded. Hyperpolarizing potential steps, each about 30 msec duration were also applied to the post-synaptic membrane, and the PSC was recorded for each step. The declining phase of the PSC was fitted to the logistic or growth curve. The rate constant in the equation did not appear to be potential dependent. Cooperativity between the closed and open channels in the post-synaptic membrane could account for the fit to the logistic equation.

Significance to Biomedical Research and the Program of the Institute: The sizes of the various permeant ions might give the dimension of the conducting pathway. The interaction of the various chemical and pharmacological agents may produce a pharmacological effect. Under these conditions, it appears that the nerve membrane may be altered. Any changes in the axon membrane might possibly give information as to the site of the particular conducting pathway. Therefore, these studies on the giant axon are making it possible to get some kind of insight in the mechanism of the excitable membrane.

Proposed Course of the Project: The study of "sodium substitutes" and especially "potassium substitutes" of the quaternary-ammonium ions and their various analogs and others will be continued combined with chemical analysis of the membrane. Naloxone, a morphine antagonist, which has to be applied internally will be studied as soon as the spring Myxicola arrive, making internal perfusion or injection much simpler. Studies on scorpion venom, heavy metals (lead, etc.), Saxitoxin and Tetrodotoxin comparisons leading to dose response curves and a prediction as to the number of Na conducting channels as well as Batrachotoxin will be resumed. It is planned to study other chemical and pharmacological agents such asesthetics, tannic acid, pronase and other enzymes. It is hoped that these studies will in

fact be much simpler with a computer controlled voltage clamp with its attendent analysis. Internal perfusion and injection will be pursued until it is as reliable as it is in squid axon.

Honors and Awards:

Leonard Binstock became a member of the Society of General Physiologists; Daniel L. Gilbert became a member of: American Society for Pharmacology and Experimental Therapeutics, International Society for the Study of the Origin of Life, Society for Neuroscience.

Publications:

Henkin, R. I., Gilbert, D. L., Stillman, I., and DiPolo, R.: Ineffectiveness of adrenocorticosteroids and adrenocorticotropin in altering Na-K currents in squid giant axon. Experientia 29: 555-556, 1973.

Serial No. NDS(I)-74 LB/OC 2089
1. Biophysics
2. Office of the Chief
3. Pethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electrical Fluctuations in Excitable Cells

Previous Serial Number: None and incorporating Serial No. NDS(I)-72 LB/OC 1948

Principal Investigators: H. Lecar, Ph.D., and F. Sachs, Ph.D.

Other Investigators: G. Ehrenstein, Ph.D., B. Cox, Ph.D., and J. Shaw, M.A.

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	1.4
Other:	0.0

Project Description:

Objectives: To determine the biophysical properties of the Acetylcholine-activated ionic channel and its functional alterations produced by pharmacological agents. To measure electrical fluctuations from other excitable cells in order to estimate the elementary conductance units of ionic channels.

Methods Employed: We have studied the fluctations in conductance of voltage clamped cells, and are attempting to see single channel responses. Power spectra of membrane current fluctuations are obtained by tuned-filter methods and by on-line computation using a Fast-Fourier transform program.

Major Findings: After initial experiments on a number of excitable tissue-cultured cell preparations, chick pectoral muscle cells were found to have the best properties for studies of membrane electrical noise. A study of voltage fluctuations in the presence and absence of iontophoretically applied acetylcholine demonstrated the existence of an electrical noise component caused by the activation of post-synaptic ion channels.

A two-microelectrode point voltage clamp was then developed in order to study electric current fluctuations induced by steady iontophoretic acetylcholine (ACh) application. The voltage clamp allows the measurement of the complete power spectrum of current noise without filtering by the membrane capacitance.

The power spectrum data was fitted to a model of channel kinetics derived by us in which post-synaptic channels open randomly in response to ACh arrival and close randomly thereafter. Both opening and closing were taken to be Poisson processes. From analysis of the data, we determined the conductance of a single ACh-activated channel to be 80 pmho. Each channel carries a current of ~3pa at normal resting potentials, and stays open for an average time of 1 msec at 35°C. The data further suggest that the duration of an open-channel event increase with increasing membrane potential.

The values found for the conductance of the elementary channels of the post-synaptic membrane is consistent with the values estimated by Katz and Miledi for the frog neuromuscular junction. This unit of conductance is also close to the value determined in this laboratory for ion conducting pores induced in lipid bilayer membranes (No. NDS(I)-74 LB/OC 2090). The results of these noise studies have been reported in a recent article in Nature.

Current improvements in the experimental system include the development of a computer program to gather and analyze on-line, noise data from tissue culture cells, the development of a temperature controlled stage to allow low-temperature studies, and an analysis of the instrumental errors introduced into power spectrum by a bandwidth limited voltage clamp.

Theoretical studies were made on the relevance of our findings on the ACh channel-receptor to chemoreception in general, and on the feasibility of observing single-channel fluctuations (as we have done experimentally with the synthetic bilayer).

Significance to Biomedical Research and the Program of the Institute: Chemically mediated synaptic transmission is the most important aspect of nervous interaction and understanding of channel mechanisms is central to the study of CNS function. Noise and fluctuation techniques also have more general applicability to the study of membrane excitation.

Proposed Course of the Project: The fluctuations studies on the ACh channels are being continued using on-line computer techniques. We now will study the effects of temperature, voltage and several anesthetics on the conductance and persistance of the channels. Experiments are also planned to observe the response of individual channels.

Honors and Awards: None

Publications:

Sachs, F. and Lecar, H.: Acetylcholine noise in tissue culture muscle cells. Nature New Biology 246: 214-216, 1973.

Serial No. NDS(I)-74 LB/OC 2090
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Lipid Bilayer Membranes: Simulation and Molecular Description of Excitability

Previous Serial Number: None and incorporating Serial No. NDS(I)-65 LB/OC 1240

Principal Investigators: G. Ehrenstein, Ph.D., H. Lecar, Ph.D.,

Other Investigators: R. Blumenthal, Ph.D., R. W. Albers, Ph.D., R. Latorre, Ph.D.

Cooperating Units: Laboratory of Theoretical Biology, NCI, NIH

Laboratory of Neurochemistry, NINDS, NIH

Man Years:

Total:	1.6
Professional:	1.6
Other:	0.0

Project Description:

Objectives: To study the mechanisms for ion transport in lipid bilayer membranes doped with ionic channels or carriers. To develop the lipid bilayer membrane as an assay for ion-conductance-inducing materials isolated from biological membranes. The ultimate goal of the lipid bilayer project is the elucidation of the molecular mechanism of membrane excitation and active transport.

Methods Employed: Lipid bilayer membranes are formed from natural membrane extracts, oxidized cholesterol, or purified phospholipids. A small quantity of lipid dissolved in a hydrophobic solvent is placed in an aperture between two electrolyte solutions, and spontaneously thins to form a bilayer with very low electrical conductance (about 10^{-10} siemens). Ion-conductance-inducing material is added to the solution on one side of the membrane, and spontaneously incorporates into the membrane. The electrical properties of the doped membrane are then studied by voltage clamp techniques. The materials added to the bilayer have been primarily EIM (a protein of bacterial origin which forms ionic channels in the membrane) and material isolated from the Na-K ATPase system of electric eel.

Major Findings: We have previously shown that EIM forms ion-conducting channels, and that in oxidized cholesterol each channel has two conductance states with conductances of about 4×10^{-10} and 0.8×10^{-10} siemens. We have now determined the kinetics of transitions between the two states by two independent methods. In one method we analyzed the statistics of transitions of a single channel between the two conductance states, and in the other method we observed the time course of approach to a steady state distribution of conductance states for a many-channel membrane. The two methods gave similar results: the transition rate from the low to the high conductance state decreases exponentially with voltage and the transition rate from the high to the low conductance state increases exponentially with voltage.

We have also shown that the material isolated from Na-K ATPase forms channels in lipid bilayer membranes and that the formation of these channels requires the presence of sodium.

Significance to Biomedical Research and the Program of the Institute: Our work on EIM comprises the first and most direct demonstration of the existence of gating channels (channels that open or close in response to membrane potential). The kinetic results also offer insight into the gating mechanism. The exponential dependence of transition rate on membrane potential suggests a linear change in free energy with electric field, and hence a change in dipole moment. Furthermore, the magnitude of the dipole moment change (about 600 debyes) suggests a large movement of the gating channel molecule relative to the membrane. This probably means that the electric field drives the channel molecules into the membrane for the high conductance state and back towards the membrane surface for the low conductance state. The kinetic results have also shown important similarities between properties of ionic channels in EIM-doped bilayers and ionic channels in axon membranes. Both systems show the same sigmoidal dependence of conductance on voltage and the same bell-shaped dependence of time constant on voltage. These similarities may well be based on similarities in mechanism.

The significance of the Na-K ATPase results is that the pumping mechanism seems to occur through ionic channels and that the specificity of the incorporation of these channels to Na may be the result of a chemical grouping with an affinity for Na. This would explain the enhanced Na permeability of the pump in the non-denatured biological membrane.

Proposed Course of the Project: We plan to study inactivation in the EIM-doped bilayer. In particular, we want to test whether voltages of opposite sign might drive channels out of the membrane in opposite directions. This is a possible analog of the inactivation process observed in biological membranes. We also plan to investigate the influence of different lipids on channel properties, particularly transition rates between conductance states. Furthermore, we would like to correlate any lipid-dependent effects with viscosity measurements determined by spin labelling experiments on multilayers.

Honors and Awards:

G. Ehrenstein was appointed Chairman of the 1975 Gordon Conference on "Biological Interaction and Transport".

Publications:

Ehrenstein, G., Blumenthal, R., Latorre, R. and Lecar, H.: Kinetics of the opening and closing of individual EIM channels in a lipid bilayer. J. Gen. Physiol. (In Press).

Serial No. NDS(I)-74 LB/OC 2091
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mathematical Modeling

Previous Serial Number: None and incorporating Serial No. NDS(I)-62 LB/OC 936

Principal Investigators: R. FitzHugh, Ph.D., and H. Lecar, Ph.D.

Other Investigators: W. J. Adelman, Jr., Ph.D., K. S. Cole, Ph.D., G. Ehrenstein, Ph.D., R. J. French, Ph.D., R. E Taylor, Ph.D., F. Bezanilla, Ph.D., J. Vergara, Ph.D.

Cooperating Units: Laboratory of Neurophysiology, NIMH, NIH

University of Rochester, Medical School, Rochester, New York

Man Years:

Total:	2.9
Professional:	2.9
Other:	0.0

Project Description:

Objectives:

a. Anodal break excitation: To explain theoretically the rapid increase in anodal break threshold with increasing temperature.

b. Modifications of Hodgkin-Huxley model: To explain the effects of potassium ion accumulation on repetitive firing in axons.

c. White noise analysis: To calculate the response of nerve membranes to white noise input, as a method of measuring impedance and for predicting the properties of random firing.

d. New computer programs for nerve models: To increase the convenience of obtaining solutions of the Hodgkin-Huxley and other nerve membrane equations.

e. Analysis of voltage clamp circuits: To improve the stability and accuracy of voltage clamp equipment.

f. Rate theory model of ionic-selective channels: To explain ion selectivity and competition in membrane channels.

g. Dielectric loss in axon membranes: To investigate the presence and nature of dielectric loss in the nerve membrane.

Methods Employed:

a, b, d. Numerical solution of differential equations by computer.
c. Statistical communication theory and nonlinear noise analysis.
e. Linear control theory and complex plane analysis.
f. Reaction rate theory and curve fitting with the MLAB computer program.
g. Analysis of published data, computation of impedances from nerve models, and solution of partial differential equations.

Major Findings:

a. Further computation, using a program developed under (d), has substantiated the earlier conclusion that in the Hodgkin-Huxley model, the threshold to anodal break excitation goes to infinity at a finite critical temperature. This behavior is clearly explained by threshold curves computed for the more general situation of stimulation by a step change of constant current from I_0 to I_1. Threshold curves are plotted in a Chweitzer diagram (I_1-I_0 versus I_0). As temperature is increased, the threshold curve rises, and its intersection point with the straight line denoting anodal break excitation goes to infinity at a critical temperature.

b. The Hodgkin-Huxley equations were modified to include an external diffusion barrier separated from the axolemma by a thin periaxonal space in which K^+ accumulates as a function of membrane activity. The alpha and beta functions for h and n were also modified according to recent experiments on squid axons. A program developed under (d) was used on the PDP-11 computer to solve the new equations. Computation of action potentials showed better agreement with the shape of the falling phase and undershoot of individual action potentials, and with repetitive trains undergoing adaptation, than are given by the unmodified equations. A preliminary report of this work by W. J. Adelman, Jr., and R. FitzHugh was given at the Annual Meeting of the Society for Neuroscience in 1973.

c. Although most of the important information about nerve excitation has been obtained by the use of the voltage clamp method, there are certain uncertainties inherent in this method. Other means of studying membrane response, such as impedance measurement, are often employed. For a linear system, measurement of the voltage response to white noise current yields similar information to impedance measurement, but often can be done more conveniently. The main emphasis of the theoretical white-noise analysis has been to test the applicability of various analytic methods to the nonlinear axon membrane.

Cross-correlograms and power spectra were calculated for the membrane potential of an axon stimulated by white noise current. The computations employed two new approximations to the Hodgkin-Huxley nerve equations. For subthreshold calculations quasilinear and linear theory were used to predict the axon response. For suprathreshold calculations, the statistical properties of random firing were described by an adaptation of the Stratonovich theory of the behavior of relays in the presence of noise. The results are in agreement with the experimental findings of Dr. R. Guttman. A joint paper with Dr. Guttman is in preparation.

d. The new programs, for the PDP-10 and PDP-11 computers, use the numerical method of C. W. Gear, which is faster than the Runge-Kutta method, and also provides efficient solution of stiff equations (those with one very short relaxation time), encountered under some conditions. One program produces action potentials for various current pulse stimuli. Another computes thresholds by searching for the point of maximum slope on the stimulus-response curve (peak membrane potential versus stimulus strength). This method permits a unique definition of threshold, even for graded responses which appear at high temperatures.

These programs have been organized so as to confine the description of the model to a few subroutines. This will simplify the modification of the model for better comparison with experimental findings which the original Hodgkin-Huxley model is not adequate, as in (b).

e. The stability of simplified voltage clamp circuits was determined by locating the poles and zeroes of the complex system control function (i) analytically, using the Routh criterion, and (ii) by computation on the PDP-11. The results were compared with actual measurements on the voltage clamp apparatus for single barnacle muscle fibers in collaboration with J. Vergara. Considerable insight was obtained into the operation of voltage clamps. Most significantly, ignoring electrode impedances, our system was unstable, but addition of series resistance stabilized it. On removing the added resistance with negative resistance compensation, the system was always more stable than with physical removal. In no case did the system blow up complete for less than 100% compensation, but with wide band amplifiers serious damped oscillations occurred for less than about 0.5 ohm cm^2.

f. From the analysis of a two barrier, one site channel model, assuming that only one ion could occupy the site at a given instant, expressions were obtained for the current-voltage relation and the reversal potential. For any two ions, the permeability ratios estimated from reversal potential measurements, and current ratios for equal driving forces, in general, are not equal. The reversal potential expression reduces to the Goldman-Hodgkin-Katz equation for certain special cases, but in the general case contains permeability expressions that are voltage-dependent. Quantitative predictions of deviations from the independence principle for current-voltage relations in single ion experiments, and of voltage-dependent action of blocking ions emerge from our examination of the model. A preliminary report entitled, "Ionic competition in axon channels -- a model", by Robert J. French, Harold Lecar and

Gerald Ehrenstein, has been prepared (Biophysical Society Meeting, Minneapolis, Minnesota, June, 1974).

g. Available experimental measurements of axon membrane impedance are limited by inadequate range of frequencies and sometimes by too widely spaced frequencies. Re-analysis of such data shows a dielectric loss with a phase angle factor of about 0.8, overlooked by previous authors. A tabulation of factors for impedance calculations for the Hodgkin-Huxley model (using MLAB) has been used for predicting the form of experimental impedance curves, as well as for the response to noise.

The constant phase angle capacitor circuit with shunt resistance can be mimicked theoretically by the input impedance of a semi-infinite tapered (Bessel) transmission line. It is not certain whether this physical model of the constant phase angle capacitance is of significance in explaining this phenomenon as observed in the nerve membrane. It provides a geometric or spatial explanation, in contrast to earlier attempts to derive this phenomenon from a distribution of relaxation times.

Significance to Biomedical Research and the Program of the Institute: Mathematical modeling of membrane excitation and the ion permeability changes underlying excitation provides a means for using experimental findings on simple nerve preparations to predict physiological phenomena which may not be amenable to controlled experiment. For example, the ion accumulations and depletions, which can be studied experimentally for the giant axon, provide the basis for modeling of accommodation and repetitive bursts of activity in nerve tissue. Thus the kind of modeling reported here provides a factual physiological basis for the more abstract neural modeling needed to explain the more complex phenomena of the nervous system.

Proposed Course of the Project:

(a) Further topics to be studied, for comparison between anodal break and cathodal make excitation, are the effect of temperature on the sharpness of the threshold phenomenon, and strength-duration curves for finite stimulus pulses.

(b) Further modifications of the Hodgkin-Huxley model are needed to permit theoretical predictions of experimental result, leading to a better understanding of nerve function.

(c) The theoretical models developed for the analysis of random spike trains and for repetitive firing give rise to analytical expressions for axon firing frequency as a function of various Hodgkin-Huxley parameters. This aspect will be pursued further as part of a study of repetitive firing.

(d) If further interactive programs for solving nerve model equations under various conditions can be written, such as for the propagated impulse and the computation of conduction velocity, they should be useful to investigators who do not wish to spend time doing such programming themselves.

(e) A computer program has been written for more complicated voltage clamp systems, including electrode impedances and some commonly used compensating schemes. Further analysis of such systems is planned.

(f) Experimentally, several possibilities are open. Using modifications of internal pH to block the sodium current in squid axon it should be possible to determine whether the site position for H^+ ion block is the same regardless of which side the H^+ ions enter the channel. Further measurements of time constants for current buildup and decay are needed to determine whether the gating processes and selectivity mechanisms in the axon are independent, as has been assumed to date in this work. On the theoretical side, we hope to determine from the selectivity sequences exhibited by the axon channels, whether we can draw any unique conclusions about the energy barrier profiles. More instantaneous current-voltage data will probably be needed for this analysis.

(g) Plans for extended experiments and analysis in collaboration with S. Takashima of the University of Pennsylvania, in 1974 are developing.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LB/OC 2092
1. Biophysics
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Subcellular and Extracellular Structures Associated with Nerve and Muscle

Previous Serial Number: None and incorporating Serial No. NDS(I)-72 LB/OC 1947

Principal Investigators: W. J. Adelman, Jr., Ph.D., K. S. Cole, Ph.D., and F. Sachs, Ph.D.

Other Investigators: R. Latorre, Ph.D., D. L. Taylor, Ph.D., and R. V. Rice, Ph.D.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Mass.

State University of New York at Albany, Albany, New York

Carnegie-Mellon University, Pittsburgh, Pa.

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description:

Objectives:

(1) To investigate the structure of intracellular water in single muscle fibers.

(2) To examine certain proteins in the axoplasm of nerve fibers.

(3) To determine if membranes can be formed from cytoplasmic constitutents.

(4) To investigate the anatomical dimensions of the Schwann cell layer external to the axon membrane and predict some of its physiological properties.

Methods Employed:

(1) The basic techniques for probing water structure with a spin label making use of electronparamagnetic resonance spectroscopy have been described previously.

(2) Squid (Loligo pealei) axons were very carefully cleaned, briefly soaked in isosmotic dextrose and then blotted dry. One end of the axon was cut and the axoplasm was extruded by means of rolling on a rubber pad. Care was taken not to contaminate the axoplasm sample with any material external to the axolemma. The axoplasm was then examined electrophoretically using a sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoretic method.

(3) A surface precipitation reaction has been produced in demembranated amoeba cytoplasm and extruded axoplasm from the giant axon of the squid. The new surface formed when the pH, ionic strength, osmolarity, divalent cation concentration and temperature were controlled. This study utilized light optical, electron optical and electrophysiological equipment. Polarized light microscopy, Nomarski differential interference optics and fluorescence microscopy were used to identify the formation of a boundary at the surface of demembranated cytoplasm.

(4) Squid (Loligo pealei) giant axons were prepared and cleaned of loose connective tissue. These axons were fixed with a variety of fixatives (gluteraldehyde, osmium tetroxide, or a mixture of paraformaldehyde, acrolein, and gluteraldehyde) in sea water and in 10% hyperosmotic sea water. Several concentrations of these fixatives were tried. When osmium tetroxide was not the fixative, the preparations were subsequently stained with OsO_4. After embedding, both cross and longitudinal thin sections of the giant axons were cut. These sections were examined in the electronmicroscope either at the Marine Biological Laboratory or at Carnegie-Mellon University. From the electron micrographs the following measurements were made: (i) radial thickness of the periaxonal space, (ii) length and width of the clefts between Schwann cells, (iii) frequency of clefts between Schwann cells.

Major Findings:

(1) Previous work has been extended to cover the temperature dependence of the spin label in model protein solutions, and a careful analysis of the effects of fast exchange on the spin label spectrum has been performed. The results supported the interpretation that the spin label undergoes fast exchange between sites of different mobility in the muscle fibers, most likely between a free solution type of environment, and a few relatively stiff binding sites. These viscosity figures should be taken as upper limits for cytoplasmic viscosity. These results indicate that cell water does not form an extensive ordered structure, and hence cannot serve as an ion exchange medium for ion maintaining ion balance.

(2) SDS gel electrophoresis of extruded axoplasm from Loligo pealei giant axons show 12 bands. The 130,000 dalton band has small satellites on each side at approximately 150,000 daltons and 100,000 daltons. The highest concentration is at 130,000 daltons just as in smooth muscle. Amino acid

composition of the material obtained from the sliced gels of each of the three bands does not correspond with any known fibrous protein, but is identical to that obtained by amino acid analysis of similar gel bands obtained from mammalian smooth muscle. It is suggested that this protein, which has been named Mesosin, is a universal fibrous protein.

(3) A surface precipitation reaction has been produced in demembranated amoeba cytoplasm and exuded axoplasm from the giant axon of the squid. The new surface formed when the pH, ionic strength, osmolarity, divalent cation concentration and temperature were controlled. Polarized light and Nomarski optics detected the presence of a refractile boundary, such as that caused by the surface of cells covered with membrane. This refractile boundary is different from the optical artifact called edge birefringence. Dyes that bind to hydrophobic sites have been applied to the preparations to identify the membrane surface. Fluorescence occurred with and without the refractile boundary, but there were quantitative differences in the time of fluorescence and the intensity. Electrical measurements gave no evidence of the presence of membranes similar to those occurring in intact giant axons and other cells.

(4) From measurements of the periaxonal space and the clefts between Schwann cells, the resistance in series with the axolemma was calculated as about $1.5 \Omega cm^2$. This value was in rough agreement with the series resistance determined from voltage clamp meassurements of the external barrier permeability to potassium ions. It was found that the thickness of the anatomical periaxonal space increased about 2x when axons were exposed to 10% hypertonic sea water. This value was also in agreement with the increase seen in the phenomenological external K^+ space obtained on voltage clamping squid axons. Therefore, it now seems that the voltage clamp measurements of the periaxonal space dimensions and the permeability of the outer barrier to K^+ ions have a real basis in the anatomic relations that satellite cells have to the axon.

Significance to Biomedical Research and the Program of the Institute: Understanding the role of these adjunct structures with respect to membrane function will expand our understanding of nerve and muscle.

Proposed Course of the Project: (1) The water structure part of this project is now complete. (2) Further work on peptide mapping of Mesosin is planned. (3) Since no electrical evidence has been produced for membrane formation, it is not clear at this time what course of action will provide information as to the surface precipitation reaction. (4) Further work is planned on chemical alteration of the Schwann sheath.

Honors and Awards: None

Publications:

Sachs, F., and Latorre, R.: Cytoplasmic solvent structure of single barnacle muscle cells studied by electron spin resonance. Biophys. J. 14: 316-326, 1974.

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Stroke

R. Wayne Albers, Acting Chief

The operations of each of the four sections are directed toward major problems in neurobiology. Each section has been able to report substantial contributions to the understanding of their chosen fields of interest. These interests range from the physical properties of isolated enzymes through the cellular physiology of brain, muscle and sea urchins. Two common objectives can be stated. One is the goal of explaining neural mechanisms in terms of molecular biology. The other goal is that of defining the biochemical basis of both intra- and inter-cellular communication systems, which exist in all cells but reach their highest level of development in nervous systems. To a large extent interest has focussed on the properties of cell membranes in recent years. Membranes are the major units of structure within the nervous system, and form a common denominator of many projects. However, problems in neurobiology often originate at higher levels of organization and accordingly at higher levels of ignorance. Problems of neurobiology may lead into almost any corner of molecular biology. The potential of the Laboratory must be measured by the extent to which problems may be pursued unhampered by restrictions of technical expertise and facilities. The Laboratory of Neurochemistry has substantially broadened its base of expertise with the development of tissue culture facilities and the incorporation of the Section on NDR.

The Section on Physiology and Metabolism has been predominantly interested in exploring the relationships between various enzymes which are generally associated with plasma membranes of eukaryotic cells. A particular achievement has been the demonstration of the existence of 5'-nucleotidase and Mg^{++}-ATPases in the form of ecto-enzymes in many cells. Such enzymes are oriented in the plasma membrane so as to act principally on extracellular substrates. Together with other observations, this has led to a working hypothesis that the extracellular release of adenyl nucleotides may subserve a transmitter function involving the regulation of cellular permeability or excitability.

The Section on Physiology and Metabolism maintains an interest in exploiting certain specializations of marine organisms to explore problems of neurobiology. In the past year this has led to studies of (1) 2'-3'-cyclic AMP diesterase activity in the brains of sharks and rays; (2) 5'-nucleotidase in developing sea urchin embryos; (3) fatty acid metabolism in the hearts of migrating and post-spawning salmon. The various studies provide an indication of the potential usefulness of marine organisms in neurochemical research. Further studies are proposed to utilize the post-spawning Pacific salmon as a model for rapid aging.

The Section on Neuronal Development and Regeneration has a major interest in factors which influence the growth and repair of nerve cells and of innervated cells. The four different projects reported this year are all related to this central concern.

The trophic effects of motor nerves on skeletal muscles are the subject of intensive study in this section, since this is one of the most accessible systems from the standpoint of experimental manipulation and analysis. Recent investigations have been designed to elucidate some past observations which seemed paradoxical in relation to the influence of motor nerves on the biochemical differentiation of muscle fibers. The new results indicate that the neurotrophic responses are conditioned by the phenotypic capabilities of both nerve and muscle cells. Some earlier paradoxical results can now be attributed to species variation in the distribution of phenotypically different muscle fibers within homologous muscles.

Another major concern in the interpretation of neural influences on muscle is the intervention of extrinsic factors on the biochemical profile of muscle fibers. The current report includes studies on the effects of frequency of muscle activation and of mechanical or thermal injury.

Histochemical methods can offer unique insights into the factors which play a role in cellular interactions. However, this methodology is subject to manifold experimental artifacts and must be handled with care. Several major achievements in new histochemical techniques and in the control and interpretation of established methods are reported this year. These are in areas of direct application to current research.

The survival of transplanted neurons is being studied with respect to its immunologic aspects. The present report establishes that host rejection of neurons in homografted ganglia can be suppressed by production of tolerance in the host. Neurons in these grafts not only survive, but regenerate their cell processes and establish functional connections with end organs of the host strain.

The Section on Enzyme Chemistry is reporting a continuation of its major concern with the molecular mechanism of active Na^+ transport. The central issue is the elucidation of the interactions between Na^+, K^+ and ATP with specific sites on the ($Na^+ + K^+$)-ATPase. Results of steady-state kinetic studies have been analysed in terms of the site affinities which can be derived by computer-assisted curve fitting. These studies define 3 qualitatively distinct sites of interaction of the enzyme with Na^+ and K^+. In addition, the role of these sites with respect to the phosphorylation and dephosphorylation of the transport system can be described in substantial detail. Immunological studies are reported which confirm the proposition that two different large polypeptides constitute the major components of the ATPase.

Physiological regulation of Na^+ transport appears as a major factor in the adaptation of brain to low-temperature metabolism in hibernating mammals. This regulation has been correlated with major changes in the membrane lipids associated with the ATPase.

A new project has been initiated in this section which is directed toward defining the biochemical regulation of the activation of astrocytes.

The Section on Amino Acids and Electrolytes in its reorganized form will pursue two projects, both of which are presently underway within the laboratory. These are (1) the application of tissue culture techniques to the study of neurotrophic factors, and (2) the study of brain electrolyte metabolism and related factors in hibernating hamsters. The first project was initiated this year in cooperation with the Section on NDR. The second project was initiated in the Section on EC.

Serial No. NDS(1)-61 LNC/AAC 810
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Metabolism and Functions of Free and Protein-Bound Amino Acids in Neural Tissues.

Previous Serial Number: Same.

Principal Investigator: D. B. Tower, M.D., Ph.D.

Other Investigators: Oscar Young.

Cooperating Units: None.

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied in vitro.

Methods: In addition to standard procedures utilizing slices or homogenates of neural tissues incubated under usual in vitro conditions, specific procedures and assay methods are referred to or outlined in the appropriate section of "Major Findings" below.

Major Findings:

Because of the transfer of the principal investigator to another assignment, there was no activity on this project and it was terminated.

Proposed Course:

Termination of this project.

Publications:

None

Serial No. NDS(1)-61 LNC/AAC 811
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Reports
July 1, 1973 through June 30, 1974

Project Title: Electrolytes and Energy Metabolism in Cerebral Tissues.

Previous Serial Number: Same.

Principal Investigator: D. B. Tower, M.D., Ph.D.

Other Investigators: O. Young.

Cooperating Units: None.

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To study the in vitro metabolism of electrolytes and of energy-producing cycles and compounds thereof, primarily in incubated slices of cerebral cortex and subcortical white matter (or in equivalent whole-cell preparations, such as tissue culture) and where possible to extend and evaluate such studies in vivo.

Methods: In addition to established, standard methods for preparation and incubation of slices (or homogenates) of brain tissue or cultures of specific cell lines in vitro, with use of appropriate media and indicator solutes, specific procedures and applications are referred to or outlined in the appropriate sections of "Major Findings" below.

Major Findings:

Because of the transfer of the principal investigator to another assignment, there was no activity on this project and it was terminated.

Proposed Course:

Termination of this project.

Publications:

1. Gill, T. H., Young, O. M., and Tower, D. B.: The uptake of ^{36}Cl into astrocytes in tissue culture by a potassium-dependent saturable process. J. Neurochem., in press.

Serial No. NDS(1)-74 LNC/AAC 2076
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Trophic Substances of Nerve.

Previous Serial Number: None.

Principal Investigator: T. H. Oh, Ph.D.

Other Investigators: O. Young.

Cooperating Units: None.

Man Years:

Total:	1.8
Professional:	1.0
Other:	0.8

Project Description:

Objectives: To investigate the chemical nature of trophic substances of nerve tissues. The initial objectives are to study the chemical factors through which nerve extracts can promote muscle cell differentiation and regeneration. A corollary objective is to assess tissues from various muscular dystrophies for possible defects in this mechanism.

Methods: Tissue culture methods are used extensively. Biochemical assays for enzymes and other components are employed to assess the response of muscle to trophic factors. Fractionation techniques will be developed as needed to characterize and isolate these factors.

Major Findings: Preliminary experiments have established methodology for the mass-culturing of dissociated muscle cells from embryonic chicks and newborn rats.

Myogenesis proceeds as previously described (Oh and Johnson, Exp. Neurol. 37, 360, 1972). Fully differentiated myotubes with cross-striations appear within the second week and synchronous contractions of large numbers of muscle fibers are seen in all cultures.

Significance: It is well established that motor nerves influence the growth and differentiation of skeletal muscle fibers. This is undoubtedly an important factor in normal development and perhaps also in adaptive responses of muscles to use. Recent evidence suggests that a lack or limitation of a trophic factor produced by nerve may be the cause of some forms of muscular dystrophy.

Trophic effects of nerves probably also act upon other nerves within the central nervous system. Thus the nerve-muscle system serves as a relatively accessible model for comparable CNS interactions.

Proposed Course: Whole brain and spinal cord tissues from embryonic and adult chickens and rats will be used as sources of "nerve extracts".

The morphological and biochemical patterns of differentiation for in vitro cultures of embryonic muscle cells will be established. The biochemical parameters will include cholinesterase, actomyosin, actomyosin ATPase and protein.

The effects of crude nerve extracts will be examined with respect to the patterns established for control cultures of muscle.

These observations will be used to establish a bioassay system which can be used to monitor the fractionation of the crude nerve extract. Physical and chemical properties of the trophic factors will be ascertained by this procedure.

Publications:

None

Serial No. NDS(1)-74 LNC/AAC 2077
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physiological and Biochemical Correlates of Cation Balance in Brain.

Previous Serial Number: **None.**

Principal Investigator: S. S. Goldman, Ph.D.

Other Investigators: None.

Cooperating Units: None:

Man Years:

Total:	0.7
Professional:	0.5
Other:	0.2

Project Description:

Objectives: To elucidate the biochemical basis of the regulation of cation balance in brain.

Methods: Cation balance in brain is considered to be the resultant of two categories of events: (1) anabolic processes, primarily active transport, which create a non-equilibrium distribution of electrolytes; (2) catabolic processes, such as membrane excitation and passive leaks, which permit electrolytes to redistribute toward equilibrium. These two categories can generally be distinguished on the basis of their responses to temperature: anabolic processes have high temperature coefficients while catabolic processes, as here defined, have a low temperature dependence.

The strategy employed in this research is to compare the electrolyte metabolism and related processes in warm adapted hamsters with those of cold adapted (hibernating) hamsters. To this end a stock of hibernating hamsters is maintained. Biochemical studies of the active Na^+ transport system in cold-adapted brain are carried out. Further details of these studies are reported under the Section on Enzymes.

Specific Methods. A working hypothesis of the current project is that alterations in membrane lipids are central to the cold-adaptation phenomenon. To this end biochemical analyses of membrane lipids are employed. These include thin layer and gas chromatographic methods.

Significance: Electrolyte balance is a central aspect of normal brain physiology. In addition, cerebral edema and epilepsy are major pathological problems related to electrolyte imbalance. The use of hibernation as a tool in this study also relates to the clinical use of induced hypothermia for surgical procedures. At present the use of this technique is limited primarily by the inability to control neurological damage related to cerebral edema. The hibernating animal has evidently solved this problem and incorporated the solution into its normal physiology.

Proposed Course: Studies to date have established certain changes in brain membrane lipids which ensue from hibernation. The detailed analysis of these changes will be continued. Physical chemical studies of the effect of these changes on lipid bilayer properties are proposed. Factors in the hamster physiology which bring about these changes will be investigated.

Publications:

None.

Serial No. NDS(1)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: R. W. Albers, Ph.D.

Other Investigators: M. de Mello, Ph.D.; S. Goldman, Ph.D.; D. Jean, Ph.D.; G. Koval; and A. Swann, M.D.

Cooperating Units: Dept. of Radiation Biology & Biophysics, Univ. of Rochester; Laboratory of Theoretical Biology, NCI.

Man Years:

Total:	**5.2**
Professional:	**3.2**
Other:	2.0

Project Description:

Objectives: To assess the functions, molecular structures and mechanisms of enzymes and enzyme systems which characterize neural tissues or which respond to neural influences. Current studies are directed toward elucidation of the mechanism and structure of the (Na^+-K^+)-ATPase which is the enzymatic expression of active Na^+ transport and hence of the membrane potential generating system of neural tissues.

The experimental objectives are:

(1) to obtain the enzyme in a pure form in order to assess the structure and catalytic properties of the enzyme in isolation from other factors;

(2) to study the structure of the environment of the enzyme within the cell membrane in order to assess the possible influences of other membrane components upon the function of the $(Na^+ + K^+)$-ATPase;

(3) to study the functioning of the enzyme in its normal cellular environment, in which the cytoplasmic and external aspects of the enzyme are exposed to different solutions.

Methods:

General methods - The methods at present employed in this section relate almost exclusively to experimental objectives (1) and (2) above. The general problem of purification of membranes and of membrane-bound enzymes is itself an area of intensive research at present. Accordingly new possibilities for improved methodology in the isolation of plasma membranes and the solubilization and purification of the (Na^+ + K^+)-ATPase continually arise and are the subject of continuing exploration. This becomes particularly important as our studies progress to the point where specific correlations are sought between the enzyme and neural function. Thus in most of our studies on the mechanism of the ATPase, we have used the electric organ of the fish, Electrophorus electricus, as a rich source of enzyme, with minimal problems in membrane purification.

As we attempt to extend our observations to more complex tissues such as brain, more complex isolation techniques are required to achieve a comparable state of enzyme purity. This involves more man-hours consumed in non-data-producing preliminary procedures.

Consequently, a continuing methodological strategy is to obtain as much relevant data as possible with the electric organ enzyme and to turn to more complex tissues only when the findings must be generalized or when the peculiarities of a more complex tissue are an intrinsic part of the question under study.

General techniques employed for enzyme preparation include differential centrifugation, ultrafiltration, gel permeation chromatography and preparative electrophoresis. Various techniques employing detergents and stabilizing agents are used to obtain active soluble enzymes from membranes.

Specific methods

(a) Preparative method for a soluble (Na^+-K^+)-ATPase from electric organ. This method was outlined in the last report. Several refinements have been added. These include (1) preliminary purification of the detergent and of the stabilizing agent to remove traces of Na^+ and K^+ and (2) use of a lipophilic resin to remove excess detergent. These refinements appear to be necessary to permit use of large amounts of enzyme in certain transient kinetic experiments outlined below (Koval, Albers).

(b) Preparative electrophoresis of the soluble ATPase has been employed in combination with SDS (sodium dodecyl sulfate)-electrophoresis of each fraction to assess the homogeneity of the intact enzyme (Jean, Albers).

(c) Preparative gel electrophoresis in SDS has been used to obtain quantities of the polypeptide subunits for use in structural studies and for use in the preparation of specific antibodies for the immunological studies outlined below (Jean).

(d) A method for quantitating the number of components in a mixture of polypeptides is under development. This consists of dispersing the polypeptides in SDS, introducing covalently bound chromophors, resolution of colored peptides by electrophoresis, scanning the electrophoretic pattern and obtaining the data in digital form, and finally, resolving the pattern into quantitative data on individual components with a computer program.

This method will be applied to a study of the stoichiometric relationship between the polypeptide subunits of the Na^+-K^+-ATPase (de Mello, Albers).

(e) Steady-state kinetic measurements of ATPase and the associated p-nitrophenylphosphatase have been extended to enzyme preparations from guinea pig brain and from beef brain.

The purpose of these studies was (1) to generalize certain findings previously reported for the Electrophorus enzyme and (2) to investigate the basis for some differences which we had observed to exist between the Electrophorus and mammalian preparations (Swann, Albers).

(f) Immunodiffusion and antienzyme kinetics have been measured using specific antibodies toward the active Electrophorus ATPase and toward the separate polypeptide subunits.

The purpose of this study was to determine whether the smaller of the two polypeptides is an integral part of the ATPase (Jean).

(g) A colony of hibernating hamsters has been established. The purpose of this project is to produce material for further investigation of the observation that the (Na^+-K^+)-ATPase in the brains of such animals have a lower temperature coefficient than is found in comparable preparations from warm adapted hamsters (Goldman).

(h) Chromatographic separation of brain phospholipids and cholesterol; gas chromatographic separations of fatty acids and long-chain aldehydes derived from these phospholipids.

The purpose of this study is to investigate the role of membrane lipid composition in relation to the observed alteration in ATPase of brains from cold-adapted hamsters (Goldman).

(i) Development of digital data acquisition procedures and data processing programs for use in transient kinetic studies.

The purpose of this work is to permit exploration of the possibility that the conformational changes which are postulated to be intrinsic to the operation of the Na^+ pump, may be more directly observable by stopped-flow kinetic measurements of the ATPase (Albers).

(j) The NDR section is reporting on a histochemical method for (Na^+ + K^+)-ATPase which was developed in collaboration with this section.

Major Findings:

(a) The steady-state kinetics of the nitrophenylphosphatase reaction catalysed by (Na^+ + K^+)-ATPase were interpreted in terms of a rather specific model in our previous publications. The essence of this model is that Na^+ and K^+ have physically distinct sites of interaction with the enzyme. This contradicts some proposals which have envisioned a single site with convertible cation specificity. Moreover, we obtained evidence for two different K^+ sites.

We have extended these studies to ATPase preparations from mammalian brains. The nitrophenylphosphatase activities of the preparations is qualitatively identical with the electric organ enzyme and the kinetics of Na^+ and K^+ activation can be fit to the same equation with only quantitative adjustments in parameters.

However, the mammalian enzymes are more convenient for a study of the influence of ATP and other nucleotides on the NPPase, since the synergism between Na^+ and ATP is a major phenomenon in the mammalian preparations and rather insignificant in Electrophorus.

The results of the nucleotide-NPPase study have added considerable detail to our earlier proposal without requiring any alterations of the existing features of the model.

It appears that several aspects of the model correlate both qualitatively and quantitatively with independent observations obtained by other workers who have measured Na^+ and K^+ fluxes across the red cell and the squid axon. Properties of Na^+, K^+ and nucleotide binding

sites derived from enzyme kinetics are remarkably similar to those derived from cation flux measurements in relatively intact cells.

(b) The immunological relationship of both of the polypeptides which are the major components of purified Electrophorus ATPase has been established. Antisera to whole enzyme or to either of the peptides have inhibitory properties in the $(Na^+ + K^+)$-ATPase assay system. Moreover, antibody against either of the polypeptides has been shown to combine with whole soluble enzyme.

(c) The observation that hibernating hamsters adapt to low-temperature metabolism by decreasing the activation energy of the $(Na^+ + K^+$-ATPase has been investigated in terms of the lipid composition of the membranes associated with the ATPase in warm- and cold-adapted hamsters.

Adaptation is accompanied by decreased membrane cholesterol and compensatory increases in the ethanolamine- and choline-containing phospholipids. Within the phospholipids, major changes were found in the phosphatidyl-cholines and the phosphatidalethanolamines. These changes consisted of decreases in certain saturated fatty acids and increases in certain unsaturated fatty acids.

These results support the hypothesis that membrane lipid composition can regulate the parameters of Na^+-active transport.

Significance: The $(Na^+ + K^+)$-ATPase is the biochemical expression of active Na^+ and K^+ transport in most animal cells. As such, it is the molecular basis for the development of electrical potentials across cell membranes, for the regulation of cell volume, water and electrolyte balance and a spectrum of coupled transport systems involving vital metabolites, neurotransmitters, etc.

Proposed Course:

(a) Structural studies of the stoichiometry of the subunits of the ATPase will be implemented, utilizing the methods of preparation and quantitation outlined above. In addition, molecular weight studies of soluble ATPase are contemplated in collaboration with Dr. M. Lewis of NEI.

(b) Regulatory mechanisms of the ATPase will be explored. These include (1) investigation of several reports of modulation of brain ATPase by neurotransmitters, (2) investigation of the possible role of sugar components of the enzyme in relation to its activity and integration into membrane structure. Some collaborative work in this area with A. Daniels of the Neurosurgery Branch is under consideration.

(c) Investigation of the stoichiometry between the number of available phosphorylation sites in the ATPase and the total number of large polypeptide subunits. This study is contingent upon the success of part (a).

The purpose of such a study is to investigate the hypothesis that the ATPase may exhibit the "half-of-sites" phenomenon which has been documented for a number of enzymes. If this is the case, there are at least two subclasses of this phenomenon which would have different implications for the mechanism and regulation of cation transport.

(d) Transient kinetic studies of the ATPase will be pursued using the improved preparations of soluble electric organ ATPase and some studies of the beef brain enzyme. Recent acquisition of a double-beam attachment for the stopped-flow photometer may produce the necessary stability and sensitivity to work with less pure mammalian ATPase.

The purpose of these studies will be to obtain relatively direct confirmation of the postulated allosteric transitions involved in cation pumping.

Publications:

1. Albers, R. W., Koval, G. J., and Swann, A. C.: Analysis of Na^+, K^+ and nucleotide interactions in terms of a heterotropic relaxation model for (Na^+ + K^+)-ATPase. Ann. N. Y. Acad. Sci., in press.

Serial No. NDS(1)-74 LNC/EC 2075
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical Regulation in Astrocytes.

Previous Serial Number: None.

Principal Investigator: R. W. Albers, Ph.D.

Other Investigators: D. H. Jean, Ph.D.

Cooperating Units: None.

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description:

Objectives: To elucidate the common biochemical denominator of the astrocytic response to chemical and physical insults to the brain. The principal focus of this project is the mechanism which "turns on" and sustains this response.

Methods:

General Methods - Initial requirements for the project include histological control to assess experimental procedures for producing an extensive astrocytic response. This facility is available through the cooperation of the section on NDR.

Currently available methods for producing "astrocytosis" include multiple stab wounds of the brain, section of optic nerves, experimental hydrocephalus, and the administration of toxic chemicals.

Some of these methods are suitable for studying acute glial responses by histological or histochemical techniques. The present requirement is for a method of producing relatively massive gliosis in a controlled manner.

For this purpose, the actions of two chemicals will be examined. These are reported to produce spongiform edema followed by widespread astrocytic gliosis.

Three different proteins are reported to be more or less specific components of astroglia. Electrophoretic and immunologic techniques have been developed in connection with the ATPase project which should be useful in establishing a correlation between the extent or stage of gliosis and the presence or absence of specific proteins.

Specific Methods - Since this is a new project, specific methods have not been established. Gel electrophoresis, including a method for quantitation which is now under development (see ATPase report) will be explored. The histochemical method for $(Na^+ + K^+)$-ATPase may be valuable along with conventional histology.

A common and well-recognized feature of the agents which cause the astrocytic response is damage to the blood-brain-barrier. Since this barrier (1) is apparently a property of capillary endothelia, (2) since it influences the transit of small ions as well as large proteins and (3) since astrocytes are intimately associated with capillaries, one profitable method to study this phenomenon may be to assess the influence of blood-born factors on the astrocytic response.

Major Findings: This is a new project. No data has been generated.

Significance: The astrocyte response occurs in demyelinating diseases and physical or chemical injury. If the injury is chronic a dense scar of fibrous astrocytes may form. This is sometimes associated with epileptic foci and the formation of these scars may often be a factor in the inability of central neurons to regenerated their normal connections after brain or spinal cord injury.

Proposed Course: The initial experiments will be primarily methodological as outlined above. A guiding hypothesis will be that there is a common biochemical pathway which translates a wide variety of noxious stimuli into a signal for increased mitotic rate and transformation to the fibrillar form.

Publications:

None.

Serial No. NDS(I)-63 LNC/NDR 1054
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Trophic functions of the peripheral nervous system

Previous Serial Number: Same

Principal Investigator: Lloyd Guth, M.D.

Other Investigators: R. Wayne Albers, Ph.D., Dou Huey Jean, Ph.D.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.5
Other:	0.4

Project Description:

Objectives: By studying the various responses of muscle to altered physiological demands, we are endeavoring to distinguish between those properties of muscle which are trophically controlled by the nervous system and those which are intrinsically regulated by the muscle cell.

Methods Employed: (i) Altered function in the soleus muscle of the rat, cat, guinea pig, and rabbit was induced by transecting the common peroneal nerve. This procedure inactivates the major antagonists of the soleus. (ii) The specificity of the histochemical procedure for myofibrillar ATPase was evaluated by use of substrates other than ATP.

Major Findings: (i) Transection of the rat's peroneal nerve results in an increased proportion of fibers having high myofibrillar ATPase activity, but there is no change in the histochemical appearance of the soleus muscle of the cat or guinea pig after this operation. There is a very small and variable increase in the proportion of high-ATPase fibers after peroneal transection in the rabbit. (ii) A myofibrillar deposition of reaction product occurs when inorganic orthophosphate (P_i) is used as substrate instead of ATP.

Significance: (i) The fact that muscle transformation could be accomplished in the rat by peroneal transection (i.e., without cross-reinnervation) constituted evidence against a neurotrophic regulation of muscle fiber types. However, this transformation could not be brought about

by transecting the peroneal nerve of the guinea pig or cat. Since the soleus muscle of the guinea pig and cat normally is devoid of high-ATPase fibers, it would seem that this effect of peroneal nerve transection is manifested only in muscles in which there normally is a mixture of low-ATPase and high-ATPase phenotypes (e.g., in the rat's soleus). This conclusion suggests the interpretation that the transformation is brought about by the selective enlargement of high-ATPase motor units, presumably by intramuscular collateral sprouting, and lends further support to the concept that the myofibrillar ATPase of mammalian skeletal muscle is neurotrophically regulated.

(ii) Because the actomyosin ATPase activity of skeletal muscle is rate-limiting in determining the speed of contraction, some investigators have suggested that individual fibers can be characterized as fast-twitch or slow-twitch according to the intensity of their histochemical myofibrillar ATPase reaction. However, we have now found that P_i binds nonselectively to myofibrils; consequently the reaction product from any muscle ATPase activity (mitochondrial, sarcoplasmic reticulum, and actomyosin) will give a spurious myofibrillar localization. This provides the explanation for why slowly contracting fibers that are densely populated with mitochondria, such as neonatal muscle, cardiac muscle, and tongue muscle, stain intensely by the myofibrillar ATPase method. For this reason histochemical observations must be interpreted more cautiously, and one cannot infer that the intensity of reaction and speed of contraction are directly related.

Proposed Course of Project: To be continued during the coming year.

Honors and Awards: None

Publications:

Guth, L.: Fact and artifact in the histochemical procedure for myofibrillar ATPase. Exp. Neurol. 41: 440-450, 1973.

Serial No. NDS(I)-66 LNC/NDR 1303
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Elucidation of factors influencing (regulating) the characteristics of skeletal muscle sensori - motor components as revealed by histochemical and other analysis

Previous Serial Number: Same

Principal Investigator: Herbert Yellin, Ph.D.

Other Investigators: Danny A. Riley, Ph.D.

Cooperating Units: None

Man Years

Total:	2.2
Professional:	2.0
Other:	0.2

Project Description:

Objectives:

1. To determine whether the enzyme histochemical properties of most mammalian skeletal muscles are neurally regulated. Particular emphasis will be placed on the motorpools of highly specialized muscles and on muscles supplied by cranial rather than spinal nerves.

2. To distinguish those factors responsible for the variability in histochemical conversion of cross-reinnervated skeletal muscle fibers.

3. To implement a system for studying the interaction of nerve and muscle cells under easily observed and readily controlled conditions.

Methods: Frozen sections of control and experimental tissues were stained for mitochondrial succinic dehydrogenase, as well as for alkali- and acid-stabile ("myofibrillar") adenosine triphosphatases (ATPases). These procedures were applied in the following studies:

1. In rats following surgical transposition of the hypoglossal (cranial nerve XII) and phrenic nerves (ipsilaterally) to the acutely denervated sternomastoid muscle. The hypoglossal nerve controls the "red" intrinsic musculature of the tongue, whereas the phrenic nerve activates the

"mixed-fiber type" musculature of the diaphragm. The recipient sternomastoid muscle normally exhibits a muscle fiber population typical of "pale" muscles found elsewhere in the body. The reinnervated muscles were assessed 4 to 16 months postoperatively.

2. In guinea pigs, rats, and several other species, typical "red" and "pale" hindleg muscles of the posterior compartment were studied following cross-reinnervation in conjunction with various physical stresses, or after the denervation of antagonistic muscles.

Major Findings:

1. Cross-reinnervation of the sternomastoid muscle with either the phrenic (respiratory) or hypoglossal (tongue) nerve results in the establishment of a functional connection between these innervations and the sternomastoid muscle (evident from visible spontaneous contractions of the reinnervated muscles, as well as by evoked responses subsequent to indirect stimulation through the appropriate donor nerves).

The histochemical nature of the reinnervated sternomastoid muscles depended upon the enzymes studied. Cross-reinnervation by either the phrenic or hypoglossal nerves resulted in increased mitochondrial content of the sternomastoid muscle commensurate with the particular nerve employed. This alteration was presumably a response to the greater frequency of activation of this muscle. However the alkali- and acid-stabile ("myofibrillar") ATPase reactions of the reinnervated sternomastoid muscle fibers were only marginally affected by the alien nerve supplies.

2. The effect of cross-reinnervation of mechanically or thermally injured muscle is currently being assessed by Dr. Danny A. Riley in his laboratory at the University of California at San Francisco.

3. Initial efforts at producing viable nerve-muscle preparations.

Significance:

1. Most motorpools throughout the neuraxis are comparable in their ability to regulate the enzyme histochemical properties related to the energy-deriving systems of skeletal muscle (oxidative vs. glycolytic metabolism, etc.). However the ATPases associated with the myofibrillar proteins appear, on a qualitative basis, to be far less susceptible to alteration as a result of alien innervation alone. Factors in addition to neural reprogramming may be necessary to permit full expression of a nerve's regulatory capabilities.

2. Awaiting Dr. Riley's findings.

3. The in vivo preparations usually utilized in the study of nerve-muscle interactions require reconstruction of on-going events from

serial studies conducted on many animals. In these instances the results may vary because of differences between animals. An effort is being made to develop an experimental model that will enable continuous observation of tissue interactions within each subject, and permit some regulation of the environment under which these tissue interactions are occurring.

Proposed Course of Project: To continue these studies (at the subcellular and cellular level) on the interaction of nerve on skeletal muscle and of muscle on nerve. In this way we hope to better understand the interaction and ultimate effect of neural programming, physical stresses (stretch, tension, etc.), humoral, and other factors on the definitive character of the skeletal muscle fiber.

Honors and Awards: None

Publications:

Estavillo, J., Yellin, H., Sasaki, Y., and Eldred, E.: Observations on the expected decrease in proprioceptive discharge and purported advent of non-proprioceptive activity from the chronically tenotomized muscle. Brain Res. 63: 75-91, 1973.

Riley, D. A.: Histochemical changes in ATPase activity during regeneration of adult skeletal muscle fibers. Exp. Neurol. 41: 690-704, 1973.

Yellin, H.: Regional differences in the contractile apparatus of intrafusal muscle fibers. Amer. J. Anat. 139: 147-152, 1974.

Yellin, H.: Changes in fiber types of the hypertrophying denervated hemidiaphragm. Exp. Neurol. 42: 412-428, 1974.

Serial No. NDS(I)-68 LNC/NDR 1586
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Trophic function of transplanted neurons; demonstration of neurotrophic capacity of homografted neurons in immunologically tolerant recipients

Previous Serial Number: Same

Principal Investigator: Andrew A. Zalewski, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objective: The prolonged survival of a homograft often depends upon whether the donor and host are compatible with respect to those antigens determined by the major histocompatibility gene locus of the species. This fact was demonstrated in studies with homografts of ganglia wherein it was found that neurons survived in 35 day homografts in rats compatible at the major histocompatibility locus whereas they were rejected in grafts between histoincompatible animals. However, with time even neurons transplanted to histocompatible hosts began to succumb to an immune reaction so that after 100 days considerably fewer neurons were present than at 35 days. Thus, it was clear that if prolonged or permanent survival of neurons in homografts was to be realized some form of immunosuppression would have to be employed. The present studies were performed to determine whether the immune rejection of neurons could be prevented as well as to see if those neurons which might survive after immunosuppression could regenerate their nerve fibers and establish a functional innervation.

Methods Employed: The type of immunosuppression used in the present study is referred to as "tolerance-induction." Brown Norway (BN) and Lewis (LE) rats were used; these animals exhibit major histoincompatibility and reject neurons in ganglia homografts exchanged between them within 35 days. To induce tolerance neonatal BN rats were injected intravenously with 30×10^6 bone marrow cells obtained from (BN x LE)F-1 hybrids. The treated

BN rats were allowed to age 140 days at which time they received a Lewis homograft of the sensory vagal nodose ganglion, one of which was placed into the anterior chamber of the eye or implanted into the sternomastoid muscle. In another series of BN treated animals a tongue graft from BN donors was combined with the Lewis nodose ganglion in the eye. This was done in order to determine if surviving LE neurons could regenerate their nerve fibers into the BN tongue graft and cause the regeneration of taste buds. As control procedures, LE ganglia were transplanted to untreated (normal) BN hosts and BN tongue grafts were placed into the eye alone (i.e., without adding a nodose ganglion).

Major Findings:

1. All neurons in Lewis ganglia were rejected by untreated (normal) BN rats.

2. Many neurons survived over 100 days in all ganglia (intraocular or intramuscular) transplanted to neonatally bone marrow-treated (i.e., tolerant) BN rats.

3. No taste buds were present in BN tongue grafts that were placed into the eye alone.

4. Regenerated taste buds were found in 8 of 10 BN tongue grafts which were combined with grafts of LE ganglia in tolerant BN rats.

Significance:

1. The present findings demonstrate that the immune rejection of neurons in homografts can be prevented by rendering the host "immunologically tolerant."

2. The results also show that neurons in homografts in tolerant recipients are functional in that they can regenerate their nerve fibers and establish an innervation (i.e., induce taste bud regeneration).

Proposed Course of Project: Neuronal transplantation studies will be continued in order to answer some of the following questions:

1. How long can neurons survive in immunologically tolerant recipients. So far we have studied grafts up to 100 days. We need to know if tolerance is a permanent state of immunosuppression or if it at some point is abrogated?

2. Can adult animals be made immunologically tolerant. If tolerance can be achieved in adults the method will have wider applicability?

3. Do tolerant animals exhibit "cross tolerance." Will an animal made tolerant to the genotype Ag-B^1 also be tolerant to an Ag-B^6 genotype? Here

we want to know if tolerance is genotype specific or is it immunosuppressive for other related genotypes?

4. Can other forms of immunosuppression (e.g., ALS serum, steroids) permit neuronal survival and nerve regeneration? The combination of tolerance and non-specific drug immunosuppression may be needed if tolerance fails? In addition other forms of immunosuppression may be superior to tolerance?

5. Can neonatal neurons be successfully homografted? An added source of homografts (adult and now neonatal) would be available for use if neonatal neurons survived. Here we would want to show that neonatal neurons could reinnervate both neonatal as well as adult tongue grafts?

6. Can central nervous system neurons survive transplantation? We contemplate transplanting spinal cord with muscle grafts to determine if survival and functional connections can be achieved.

7. Can reflex connections be made between the homografted ganglia and the central nervous system of the host? This is a crucial experiment and can be performed by utilizing sensory or sympathetic ganglion. In the case of sensory ganglion, the peripheral nerve process of the neurons will be grown out into the tongue while the central nerve process is directed toward the central nervous system. Hopefully the peripheral process of the homografted neurons will induce taste buds while their central process will establish synaptic contact with CNS neurons. A reflex connection can be made with sympathetic neurons by allowing preganglionic sympathetic fibers of the host to form synaptic contact on neurons of homografted neurons while the postganglionic fiber of the grafted ganglia are regenerated into an appropriate end-organ such as the denervated iris of the eye.

The past and present studies are being designed to establish the immunological and anatomical conditions necessary for successful neuronal transplantation. In time neuronal transplantation may be one way of treating diseases that cause neuronal death.

Honors and Awards: None

Publications:

Zalewski, A. A. and Silvers, W. K.: Trophic function of neurons in homografts of ganglia in immunologically tolerant rats. Exp. Neurol. 41: 777-781, 1973.

Zalewski, A. A. and Silvers, W. K.: Survival of neurons in homografts of ganglia in adult rats neonatally treated with bone marrow or lymph node cells. Anat. Rec. 178: 243-252, 1974.

Serial No. NDS(I)-74 LNC/NDR 2074
1. Neurochemistry
2. Neural Development & Regeneration
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regeneration in the central nervous system

Previous Serial Number: None

Principal Investigator: Lloyd Guth, M.D.

Other Investigators: R. Wayne Albers, Ph.D.

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.5
Other:	0.4

Project Description:

Objectives: To study mechanisms influencing axonal outgrowth and factors controlling functional restitution following neural injury.

Methods Employed: (i) Development of a new technology for the preservation of central neural tissues which permits the development and application of enzyme histochemical methods that provide information about the functional as well as the structural integrity of the nervous system. (ii) Evaluation of functional recovery of diaphragmatic function after high spinal hemisection in the guinea pig.

Major Findings: (i) When the brain or spinal cord is perfused by the usual methods and frozen shortly thereafter, the neuronal cell bodies and nuclei appear severely distorted, but by perfusing with polyvinyl pyrrolidone, this severe artifact can be avoided and the tissue sufficiently well-preserved for light microscopy. The tissue preserved in this way is suitable for enzyme histochemistry, silver staining or Nissl staining. A new technique for (Na^+-K^+)-activated ATPase has been developed and validated. It selectively stains the neuropil of the spinal cord and the distal tubules of the kidney. It is K^+-dependent, ouabain-sensitive, formalin-sensitive, and is unrelated to alkaline phosphatase activity. Improved procedures for revealing choline acetylase activity and succinic dehydrogenase activity have also been applied to tissues preserved in this way.

(ii) After spinal hemisection at C1, the ipsilateral hemidiaphragm is paralyzed. If the contralateral phrenic nerve is transected at this time, the animal dies from bilateral diaphragmatic paralysis. But if the contralateral phrenic nerve is transected several weeks later, the paralyzed hemidiaphragm (on the side of the previous cord section) suddenly resumes contracting with a strength sufficient to permit the survival of the animal. This crossed phrenic phenomenon in the guinea pig, unlike the cat or other animals, requires a period of several weeks between the two operations to become manifest.

Significance: (i) One of the major impediments to the study of central neural regeneration has been the difficulties of applying new histochemical techniques to the brain and spinal cord. With this technical improvement, we can now begin to evaluate the functional and biochemical plasticity of the nervous system during normal states and after injury. (ii) Studies are in progress to evaluate the functional changes that occur following the spinal hemisection to evaluate the mechanism by which synaptic efficacy is restored to the presynaptically denervated phrenic motor neurons.

Proposed Course of Project: To be continued during the coming year.

Honors and Awards: None

Publications:

Guth, L. and Windle, W. F.: Physiological, molecular, and genetic aspects of central nervous system regeneration. Exp. Neurol. 39: 3-16, 1973.

Serial No. NDS(1)-67 LNC/PM 1480
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals.

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: A. A. Benson, Ph.D.; V. Glisin, Ph.D.; S. Patton, Ph.D.; E. A. Brown, Ph.D.

Cooperating Units: Scripps Institute of Oceanography, La Jolla, Calif.; Centre for Multidisciplinary Studies, Belgrade, Yugoslavia; Div. of Food Science, Pennsylvania State University; Mote Marine Laboratory, Sarasota, Fla.; Pulmonary Branch, NHLI.

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To exploit the great variety and abundance and the biochemical specialization of oceanic life for the study of neurobiology. In particular we have selected to study some aspects of neurochemistry in marine organisms where certain tissues, species or phenomena constitute an evolutionary amplification of normal human physiology.

Methods: Our studies have been concerned with the metabolism of plasma membranes and transmitter substances. The methodology in use is adjusted to specific requirements according to current laboratory techniques. Use is made of radioactive precursors, i.e. labeled biogenic amines are introduced for studies of their metabolism in vivo or in vitro. Common enzymological approaches are used when appropriate, especially in drug metabolism, toxicology, and when the effects of putative transmitters on metabolic pathways is investigated.

Experimental animals or organisms used: various kinds of Selachians, Torpedinidae, several species of Teleosts, in particular Electrophorus electricus and Pacific salmon (Oncorrhyncus spp.); small crustaceans, Ecchinoderms, Aplysia. Emphasis has been placed on the study of

membranes, electric organs and nervous tissues or tissues which are of interest to our principal program on structure and function of excitable membranes.

Major Findings: 2',3'-Cyclic adenosine monophosphate 3'-phosphoesterhydrolase is an enzyme which appears to have a preferential localization in the brain. Although this enzyme has been associated with the development of myelin (and also with demyelinating processes) its actual function has remained obscure. It is obvious that 2',3'-cyclic AMP is not the natural substrate and it was shown elsewhere that another possible candidate, D-inositol 1:2 cyclic phosphate, was hydrolyzed by a phosphodiesterase which could clearly be distinguished from this enzyme. We had the opportunity to conduct comparative studies on the 2',3' cyclic nucleotide diesterase in some marine vertebrates using a method for estimating the activity of this enzyme which was recently developed in our laboratory.

The activity of 2',3'-cyclic adenosine monophosphate 3'-phosphoesterhydrolase was determined in the brains of several elasmobranchs and teleosts by a fluorometric method using the fluorescent analogue of 2', 3'-cAMP, 1, N^6-ethenoadenosine-2',3'-cyclic monophosphate as substrate.

Enzymatic activity of lemon shark brain was optimal at a pH of 6.5 in acetate buffer and at a temperature of about 30°C. Brain enzyme activity in lemon, nurse and brown sharks was similar and was 0.17 to 0.25 mmoles/gram wet weight tissue/hour. Brain homogenates of sting rays (Dasyatis spp.) had an activity of 0.32 and 0.34, respectively, and the brain homogenates from two teleost fish assayed for 0.34 and 0.39, respectively. Assay of enzyme activity in various portions of elasmobranch brain showed that telencephalon was consistently higher than whole brain while medulla was lower; optic lobes and cerebellum exhibited intermediate activity.

We have also made use of the marine environment to extend our studies on the function of the plasma membrane enzymes to other species. In particular we have used sea urchin eggs and sea urchin embryos at different stages to study membrane ATPases and 5' nucleotidases. In contrast to the mammalian cell, it was observed that the membranes of the unfertilized egg or the developing sea urchin embryo in the early stages of development were devoid of ecto-ATPase or 5'-nucleotidase activity. Attempts to elicit changes in membrane permeability by the addition of various adenine nucleotides to such systems failed.

We made a brief feasibility study of using a new and improved method for the isolation of cellular RNA and DNA. The method (by V. Glisin) depends on a modified use of cesium chloride and promises significantly increased yields. Our main interest was in applying this method for the isolation of fish brain RNA and DNA and possibly for the isolation of the polynucleotides of single giant neurons of aplysia.

During a 3-week study period aboard the R/V Dolphin (Foundation for Ocean Research, San Diego, California) in British Columbia waters, we surveyed organ lipid composition in a number of fish species with the aid of thin-layer chromatography. The relatively high levels of triglycerides (TG) in heart muscle of migrating salt-water salmon seemed impressive and we therefore investigated the lipid composition in salmon hearts as compared to that in hearts of other local fishes. Heart TG content was also studied in post-spawning salmon (Oncorhyncus sp.), since this fish in the post-spawning condition is generally lipid-depleted and does not survive after spawning.

Triglycerides (TG) were substantially elevated in heart lipids of pre-spawning Pacific salmon as compared to other marine fish. Tracer studies with ^{14}C-palmitate showed that FFA was largely converted into TGFA in the heart of migrating salmon. In spawned-out salmon a striking reduction of TG in heart lipids revealed another facet of metabolic collapse of this species during the reproductive cycle. We suggest that FFA may not be directly available to the muscle from the plasma compartment. We further propose that plasma fatty acids are converted to TGFA in the heart muscle prior to utilization in the cardiac energy household. Whether or not this constitutes an obligatory pathway or only a metabolic shunt, mostly activated during energy crises, should be the subject of further experimentation. The migrating salmon in this case may serve as an excellent model for such studies.

Significance: These investigations emphasize the usefulness of the marine environment in the search for models of molecular biological mechanisms or diseases. In addition, comparative studies often lead to a better understanding of certain structures or functions of a biosystem. A case in point is the enzyme 2',3' cyclic AMP-3'-phosphoesterhydrolase. This enzyme was originally thought to be a myelin marker enzyme. At present its distribution is not certain, it is known to be very active in all nervous systems investigated, but its natural substrate is yet to be discovered.

Our collaborative study (with S. Patton and A. A. Benson) on the lipid metabolism of salmon heart muscle has revealed a new aspect of cardiac metabolism: namely the possibility that the major energy source of the heart muscle, fatty acids, are available to this tissue only as triglyceride fatty acids. This is in contrast to the classical notion that the main energy source of muscle is free fatty acid. We have postulated that this unusual pathway in cardiac muscle may be obligatory and constitute a so called "energy sink" which affords the heart additional protection in times of high demands or overloads.

Proposed Course: We will continue our comparative studies in the field of neurobiology and the biochemistry of plasma membranes. For the Fall of 1974 we have planned an extended field trip aboard the R/V "Alpha Helix". We hope to make further studies on the neurochemistry of the

migrating salmon, which we believe to be an excellent molecular model for the study of certain aging processes.

Honors and Awards: E. G. Trams, member of the 1973 R/V "Dolphin" Expedition to British Columbia, Canada.

Publications:

1. Trams, E. G., and Brown, E. A. B.: The activity of 2',3'-cyclic adenosinemonophosphate 3'-phosphoesterhydrolase in elasmobranch and teleost brain. Comp. Biochem. Physiol., in press.

Serial No. NDS(1)-67 LNC/PM 1481
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Composition and Metabolism of Cellular Membranes.

Previous Serial Number: Same.

Principal Investigator: E. G. Trams, Ph.D.

Other Investigators: W. Reichert, M.D.; Carl J. Lauter.

Cooperating Units: None.

Man Years:

Total:	2.7
Professional:	1.8
Other:	.9

Project Description:

Objectives: To elucidate the inter-relationship between the molecular composition of the plasma membrane and the functions that such building blocks serve in transport, bioelectrogenesis and inter-cellular communication.

Methods: Due to several recent developments in our program we have shifted emphasis from our previous methodology towards more extensive work with clones of cultured cells. Towards this end a variety of cell lines were either purchased or propagated in our laboratory. The elements of our tissue culture methodology are based on standard procedures and need not be elaborated. More space and time, however, have been needed to provide an adequate supply of cultured cell for the various phases of our investigation. At the present time the following cell lines serve as our principal resources: Neonatal Syrian hamster astrocytes (NN cells), mouse glioma clone GL 24, mouse neuroblastoma clone N-18, mouse fibroblast L929, human choriocarcinoma of the cervix (HeLa) and human carcinoma of the nasopharynx (KB cells).

Another aspect of our research required that ionic fluxes across the plasma membrane be evaluated under various experimental conditions. These studies were conducted with a variety of isotopic tracers (^{32}P, ^{42}K, ^{22}Na, ^{35}S, ^{36}Cl and ^{14}C). Some of these isotopes are fairly heavy emitters compared to our previously used materials and additional

precautions, space etc. were required to ascertain that these isotopes were handled according to the prevailing safety standards.

Some of our routine analytical methods appeared to lack appropriate sensitivity or selectivity and we have spent some time on their improvement. Currently under development is a modified method for the assay of adenosine and related compounds which we hope to extend for histochemical purposes.

Major Findings: Our investigations during the last year on the "sidedness" of plasma membrane enzymes have led us to the conclusion that a group of catalytic functions, which generally were referred to as plasma membrane marker enzymes, participate in an interrelated sequence of reactions. The first observation of major significance was that a Mg^{2+} dependent ATPase and 5' nucleotidase were located on the outer aspect of the plasma membrane. Comparative studies on a variety of mammalian cell types have led us to conclude that these two enzymes characteristically face the extracellular space (or adjacent cells) and that their function is related to biochemical events which occur on the external aspect of the plasma membrane. Further investigations revealed that these two phosphoesterhydrolases subserve an important regulatory function. At this time it would be premature to regard this system as ubiquitous in eukaryotic cells, but with the exception of the human erythrocyte and sea urchin egg we have found this transverse asymetry of ATPase - 5' nucleotidase distribution in all mammalian cells.

Additional studies and isolated reports in the literature further indicated that this enzyme distribution may play an especially significant role in excitable tissues. We have only recently come to realize that the class of ecto-enzymes which we have studied probably is part of a basic control mechanism which regulates cellular permeability and/or excitability. We are inclined to believe that we are studying a transmitter system which developed very early on the evolutionary time scale. Cholinergic and adrenergic controls may have superseded it in more highly developed syncytia, though it is conceivable that the so-called purinergic nerve system is a modification of the primordial system.

In brief: we have observed that ATP can be ejected from an intracellular pool into the extracellular compartment. This ATP translocation is probably enhanced by electrical stimulation. When ATP impinges onto the surface membranes of adjacent cells it a) induces a marked increase in permeability and b) is immediately converted to ADP by ecto-ATPase. ADP is converted to AMP which serves as a substrate for the ecto-5'-nucleotidase. The product of this reaction, adenosine, has a selective antagonistic effect to ATP and terminates further translocation of ATP. Ecto-adenosine deaminase eventually degrades this compound to inosine which has no effects. Barbiturates which can be regarded as congeners

of adenosine have a similar effect. The system seems to be insensitive to a large number of other classic metabolic inhibitors. We are currently exploring the possibility that the mode of action of barbiturates is through an adenosine receptor in the plasma membrane.

Another finding of significance is that certain glia cells appear to be peculiarly sensitive to ATP application. From these observations and from data in the literature we have formulated a working hypothesis for the study of the molecular basis of convulsive disorders.

During the course of these investigations it was found that plasma membranes were the source of an active inorganic pyrophosphatase. A series of studies on the properties and kinetics of this enzyme was undertaken. It was found that various cells exhibited the presence of either one of two forms of inorganic pyrophosphatase: a Mg^{2+} dependent enzyme or an enzyme which did not require any cations for activity. We have been unable to relate these enzymes to the above described scheme. The presence of an ATP pyrophosphohydrolase could not be demonstrated in our cultured cell lines.

Significance: We believe that recognition of the widespread occurrence of a series of inter-related phosphoesterhydrolases on the exterior aspect of the cell membrane constitutes a significant advance in our understanding of the plasma membrane. We also view it of fundamental import that these ecto-enzymes appear to participate as regulators in a physiologic control mechanism. If the mode of action of barbiturates, which has been obscure so far, can be explained on the basis of these findings, pharmacologic advances may ensue. Whether or not this investigation will lead to an understanding of the molecular biology of convulsive disorders remains to be seen; we view it as significant, however, that we were able to develop a novel working hypothesis founded on developments in basic research.

Proposed Course: We plan to extend our studies on the role of the ecto-enzymes in physiology. It is expected that the program will in part be directed towards a better understanding of the ATP-ATPase/Adenosine-adenosine deaminase control of permeability and excitability. The strategy will be to interrogate animal models of convulsive disorders. In the near future we shall initiate studies with mice prone to audiogenic seizures. Other elements of this study shall be concerned with electroconvulsive shock, development of mirror foci and analyses of excised human epileptic foci.

Publications:

1. Trams, E. G.: A rapid fluorometric assay for 2',3'-cyclic adenosine monophosphate 3'-phosphoesterhydrolase. J. Neurochem. 21: 995-997, 1973.

2. Trams, E. G., and Lauter, C. J.: On the "Sidedness" of plasma membrane enzymes. Biochim. Biophys. Acta, in press.

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Perinatal Physiology, IR
National Institute of Neurological Diseases and Stroke

Ronald E. Myers, M.D., Ph.D., Chief

The Laboratory of Perinatal Physiology continues to focus upon four program areas:

1. Pathophysiology and pathogenesis of perinatal brain damage.
2. Pregnancy abnormalities which adversely affect brain development.
3. Neuropathologic aspects of pulmonary and circulatory diseases.
4. Brain mechanisms underlying emotion and social behavior.

Four distinct patterns of brain injury have been produced by asphyxia to the term monkey fetus. One pattern affects structures in the brain stem, e.g., the inferior colliculi, the vestibular nuclei, certain nuclei of the thalamus, etc. This brain stem pattern is produced by exposing fetuses to episodes of total asphyxia (anoxia). A second pattern affects the cerebral cortex and consists of hemorrhagic or non-hemorrhagic tissue necrosis in the early stages and of atrophic cortical sclerosis, ulegyria, or lobar atrophy late. This cortical injury pattern is produced by severe partial asphyxia (acidotic hypoxia). A third pattern affects the basal ganglia and appears as a sclerosis of the basal ganglia or status marmoratus. This pattern results from the sequential occurrence of a partial and total asphyxia (anoxia plus hypoxia). This basal ganglia injury may be associated with injury to cerebral cortex or to brain stem depending upon the severity and duration of the hypoxic and anoxic components. The fourth injury pattern, that affecting the cerebral white matter (periventricular leukomalacia or cystic white matter degeneration) appears following an a-acidotic hypoxia where a severe oxygen deprivation occurs unaccompanied by hypercarbia or significant respiratory or metabolic acidosis.

The effects of severe asphyxia or of teratogenic agents on the fetal brain differ according to gestational age. Application during late fetal stages causes only destructive lesions whereas application during early fetal life causes either gross destructive lesions leading to hydranencephaly or porencephaly or to subtle patterns of cerebral malformation including agenesis of corpus callosum, gray matter heterotopia, and pachy- or micro-gyria. Alternatively, early injury may lead to patterns of cerebral dysgenesis where overall brain development is retarded giving rise to another pattern of microcephaly, abnormal convolutional patterning, or, indeed, to no apparent structural abnormality but with severe functional deficits. These studies indicate that a diversity of behavioral, intellectual, and neurologic abnormalities including infantile autism, hyperactivity, a variety of syndromes of minimal brain damage, mental retardation, cerebral palsy, etc., may be caused by insults which act during early fetal life.

Intra-amniotic hypertonic saline injections are used to terminate midgestational human pregnancies. Current studies show that fetal death and expulsion under these circumstances are caused by a congealing of blood and stasis of flow in the small umbilical blood vessels which pass over the chorionic plate.

In earlier fetuses, the resultant asphyxia is total and leads to anoxic death within 15-30 minutes. In older fetuses, the larger caliber of the vessels and their higher volume blood flow prevents a gel transformation of the blood and protects the fetus from asphyxia. Some fetuses of intermediate gestational age survive and are brain damaged. A corresponding stasis of flow occurs in small blood vessels of the decidua and uterus and leads to uterine wall damage and Couvelaire uterus. Thus, in addition to the risk of fetal brain injury with survival, the intra-amniotic instillation of hypertonic saline also may produce structural abnormalities of the uterine wall and higher incidences of sterility and of later pregnancy abnormalities including uterine rupture, placenta previa, etc. The local tissue necrosis and thromboplastin release caused by the stasis of blood flow produces generalized intravascular coagulation and the risk of maternal shock and postpartum death.

Studies with catecholamines and adrenergic blocking agents have revealed regulatory roles of major importance for the sympathetic nervous systems of both the pregnant female and of the fetus with respect to the fetal status in utero. For example, the blood flow to the uterus and the placental intervillous spaces are directly regulated by the maternal sympathetic tonus. Thus, excessive maternal sympathetic stimulation (such as psychologic stress) may cause fetal asphyxia with brain injury or death. The effects of prostaglandin $F_{2\alpha}$, prostaglandin E, and of oxytocin on uterine activity and on the state of fetal oxygenation have been examined. All three agents may damage the fetus when their use leads to excessive uterine activity or diminished intervillous space perfusion by virtue of uterine vasoconstriction. The relative safety of prostaglandin $F_{2\alpha}$ and of oxytocin in the induction of labor is currently under investigation.

The effects of different degrees of asphyxia (hypoxia) on the fetal cardiovascular performance and brain integrity have been clarified. The fetus can sustain even moderate degrees of asphyxia (O_2 contents down to 4 vol%) with no short- or long-term effects. Indeed, only when the O_2 content decreases below 1.5 vol% does fetal brain injury or death occur. The severity of asphyxia required to produce brain injury is close to that required to produce death.

Carbon monoxide inhalation by the pregnant monkey leads to hypoxia of the fetus and, ultimately, fetal brain damage and death. This hypoxia primarily relates to the preemption by CO of the hemoglobin binding sites for oxygen both in the maternal and the fetal blood streams. This occlusion of hemoglobin-oxygen binding sites produces an impaired oxygen-carrying capacity. However, only small quantities of CO reach the fetus because of its extremely low partial pressures in the maternal blood. The fetal brain injury produced is characterized by a more prominent brain swelling and a greater tendency for basal ganglia injury than with fetal hypoxia produced in other ways.

Carbon monoxide inhalation by adult rhesus monkeys produces symmetrical zones of hemispheral white matter necrosis. This pathology resembles that which may be seen in the human under the same circumstances. However, such white matter injury occurs under a variety of other circumstances and represents one of the important patterns of nervous system reaction to injury. Studies with the monkey fetus support the view that this pattern of injury

after exposure to severe hypoxia (or hypoergia) where there is a minimal accumulation of CO_2 and a relative preservation of normality in hydrogen ion concentration (a-acidotic hypoxia). This fits well our observation of the prominence of white matter injury also following carbon monoxide inhalation, and cyanide infusion. In all these circumstances, CO_2 accumulation fails to occur and the disturbances of hydrogen ion homeostasis are minimal.

Studies of juvenile monkeys using a variety of models of brain injury have indicated a poor correlation between the absolute values of energy deprivation and the occurrence of and severity of brain injury. A similar variable relation exists between the severity of oxygen deprivation and the degrees of alteration in cardiovascular performance. That is, the heart rate decreases and lowering of blood pressure expressed in different animals vary considerably relative to the partial pressures of oxygen in their circulating blood.

Juvenile rhesus monkeys have been subjected to controlled episodes of hypoxia where the pO_2s have been regulated from 10-30mm Hg. These animals can sustain pO_2 levels as low as 20mm Hg for varying lengths of time without sequelae. However, when the pO_2s are reduced below 20mm Hg, the animals show varying degrees of cardiovascular and nervous system decompensation and may die in cardiogenic shock or show brain pathologic changes.

To more fully characterize the alterations in cardiovascular performance exhibited by rhesus monkeys subjected to energy-deprivation states, a computerized system has been devised with the collaboration of the Laboratory of Applied Studies of the Division of Computer Research and Technology which enables a constant monitoring of such parameters as cardiac contractibility, cardiac output, and peripheral vascular analog signals of blood pressure, left ventricular pressure, and EKG are recorded directly on magnetic tape. This tape is then digitized and used as input to the IBM-360 computer system. From this data and appropriate programming, the computer can derive all multiple indexes of circulatory function at any time during the experiment. The accuracy and reproducibility of these deviations permit a more precise analysis of pressure waveforms compared with the usual direct measuring analog methods. The effects of frequency response of the recording system, of phase shifts introduced by analog differentiators, and of various components of a Fourier analysis of the pressure waveforms on the derived indexes of contractility have been defined. A detailed account of the cardiovascular response to severe hypoxia and to anoxia has also been presented. Measurements are being examined from the recovery phase after such insults to determine whether animal survivability can be predicted on the basis of a detailed knowledge of the cardiovascular responses to the insult substantially before the final outcome has otherwise become clear. Efforts are also being made to define more precisely the determinants of the final patterns of brain pathology produced.

Studies on the brain pathologic effects of severe arterial hypotension (to 25mm Hg) in the juvenile rhesus monkey have continued. During the first five minutes the cerebral blood flow rapidly decreases to levels of 10-20% of normal. Thereafter, the flow rate recovers slowly to 20-30% of normal. At the same time, the highly differential patterns of normal flow rates according to brain structure are dramatically altered in the direction of greatly diminished differences in such differential patterns. Thus, even the normally occurring wide differential in flow rates between gray and white matter (10:1)

decreases during hypotension to a 3:1 ratio. The hypotension also produces significant increases in impedance of cortex and decreases in amplitude and frequency of electroencephalogram. The animals show bradycardia (by 25-30%) and decreases in amplitude of T wave of the electrocardiogram. A respiratory depression develops during the latter half of the 30-minute episodes leading to apnea. The serum and cerebrospinal fluid glucose contents increase due to catecholamine stimulation. Intracranial pressure shows minimal changes.

Following restoration of blood pressure, the cerebral blood flow rapidly restores to normal or supranormal rates (to 50-150%). After 15 minutes, however, the animals divide into two groups: those where the cerebral blood flow remains at near-normal rates and those where the flow again decreases to 50-70% of normal. These two modes of responding then endure for many hours, the one group surviving with no brain pathology and the other dying with swollen brains (vide infra). The cortical impedence also recovers to normal values by 5-30 minutes following blood pressure restoration whereas the EEG recovery required 60-120 minutes. The cisterna magna fluid and serum glucose levels showed further increases in both groups following restoration of blood pressure and plateaued by 60 minutes into the recovery period. However, beginning at 60 minutes in the cisterna magna fluid and at 120 minutes in the serum, the glucose levels of the animals which went on to die increased beyond the values of those animals which were to recover. The non-survivors also began showing major increases in impedance values and flattening of the EEG beginning at 150 minutes.

Those animals which maintained normal cerebral perfusion, EEG activity, impedance values, and less elevated glucose levels went on to full clinical recovery. Those animals which, following an initial restoration of blood pressure, EEG and impedance values began again to show significant metabolic and circulatory abnormalities went on to die at between 12-48 hours and showed brain swelling on brain pathologic examination. No evidences of border zone circulatory deficits appeared either during the hypotensive or the recovery phases.

Studies have continued of the changes in carbohydrate metabolism of the brain and other organs in response to asphyxia, hypoxia, and other energy deprivation states. Episodes of total asphyxia or of circulatory arrest in fetal or adult monkeys causes depletions of glycogen down to a basal level over an 8- to 10-minute period. After resuscitation, glycogen again accumulates in the brain but to supranormal levels (2X) which peaks at 12 hours. This post-insult hyperdeposition response (primarily in brain astrocytes) is all or none--all periods of asphyxia up to nine minutes fail to produce hyperdeposition whereas all periods of asphyxia beyond nine minutes (and up to 30 minutes) leads to its full expression. The hyperdeposition seems to develop in response to an induced hypometabolic state of the brain caused by the asphyctic exposure. This relation to reduced brain metabolism is confirmed by the fact that glycogen hyperdeposits in the brain during and following long periods of deep barbiturate anesthesia. Severe episodes of hypoglycemia (blood glucose levels below 20 mg%) for six hours first causes cerebral glycogen depletion followed by its hyperdeposition when the blood

glucose is restored. The glycogen hyperdeposition response shows no relation to the pathologic lesions in the brain.

Examination of various parameters of brain glycogen metabolism of newborns and adults including its maximal rate of deposition during the hyperdeposition response, its rate of breakdown during asphyxia, the minimal tissue contents achieved, the tissue content during normal storage show close similarities. Thus, the metabolism of glycogen with respect to these two developmental stages is closely comparable.

Starvation leads to maximal depletion of glycogen (90%) by 24 hours. During this time, the brain shows significant glycogen increases. Peak values of glycogen in the brain (130%) occur after 48 hours. Upon restoration of food supply, the brain and liver require 6 and 24 hours before normal glycogen levels are seen. These data suggest the brain may show an overall reduction in metabolism during starvation. This correlates with lassitude and diminished bodily activity which occurs among animals undergoing starvation. At any rate, changes in cerebral glycogen during and following starvation cannot be attributed to variations in serum glucose levels since these are maintained within normal limits.

Serial No. NDS(I)-66 LPP 1388
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Perinatal asphyxia and its CNS consequences

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Myron D. Ginsberg, M.D.
Karlis Adamsons, M.D.
Stanley B. Holstein, M.D.
Maurice Panigel, M.D.
Ingemar Joelsson, M.D.

Cooperating Units: Mt. Sinai School of Medicine, New York
University of Paris, Paris, France
Sabbatsberg Hospital, Stockholm, Sweden
University of Pennsylvania, Philadelphia, Pennsylvania
Columbia University, New York, New York

Man Years:
Total: .71
Professional: .14
Other: .57

Project Description:

Objectives: To determine the clinical and neuropathologic changes produced by asphyxia of the fetus during pregnancy or at birth and to correlate the patterns of neuropathologic change with functional alterations observed in later life.

Methods Employed: Monkey fetuses are delivered by Cesarean section and subjected to episodes of total asphyxia (anoxia) for predetermined lengths of time by placing a rubber sac over the fetal head and clamping the umbilical cord. In some instances, an effort is made to produce the maximum injury compatible with survival. Other fetuses are subjected to episodes of partial asphyxia (hypoxia) which may be produced in a variety of ways most of which are associated with an impaired intervillous space perfusion. The cardiovascular, acid-base, respiratory gas, and respiratory changes which occur during and after the asphyxia are recorded and later correlated with the clinical and brain pathologic abnormalities produced.

Major Findings: With the varying durations of asphyxia, varying severities of brain damage are produced. The most common clinical alteration observed

following anoxia is a widespread sensory loss manifested as abnormal posturing and placing reactions, altered sucking and swallowing, altered vocalization, and varying degrees of ataxia and motor weakness. With more severe damage, the animals show changes in postural tone and in use of the extremities. Total paralysis may be seen and the extremities may show a severe flexor or textensor hypertonus. Pathologically, destructive changes affect various brain stem loci and the thalamus. Damage to cortex or basal ganglia rarely occurs with anoxia.

Episodes of partial asphyxia (hypoxia) do not damage lower brain stem structures, but, instead, affect various hemispheral structures including the cortex, the hemispheral white matter, and the basal ganglia. These hemispheral patterns of brain injury closely resemble the patterns of injury observed after human perinatal asphyxia.

Significance: The intent is to reproduce the neuropathology of human cerebral palsy and mental retardation. This goal has been only partially achieved. Ulegyria, atrophic cortical sclerosis, lobar atrophy, status marmoratus, periventricular leukomalacia, porencophaly, hydranencephaly, and microcephaly have all been experimentally reproduced in a controlled fashion.

Proposed Course of Project: The effort is continuing to determine the critical parameters which lead to a deterioration of the fetus during asphyxia and to relate these physiologic alterations to the patterns of brain injury produced. Efforts are also under way to develop techniques to prevent the deterioration of the fetus when asphyxiated and to prevent or postpone the occurrence of perinatal brain injury.

Honors and Awards: None

Publications:

Myers, R. E.: Threshold values of oxygen deficiency leading to cardiovascular and brain pathological changes in term monkey fetuses. In Bruley, D. F., and Bicher, H. I. (Eds.): Oxygen Transport to Tissue. New York, Plenum Publishing Corp., 1973, vol. 37B, pp. 1047-1054.

Serial No. NDS(T)-67 LPP 1464
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry of the recovering rhesus monkey from various different states of energy deprivation

Previous Serial Number: Same

Principal Investigator: Americo Rivera, Jr., Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	2.31
Professional:	1.14
Other:	1.17

Project Description:

Objectives: To investigate the effects of energy deprivation on various constituents of the brain and vital organs of the rhesus monkey and to relate these changes to brain damage. These projects include:

a) The glycogen concentration of the tissues of newborn monkeys recovering from total asphyxia.

b) The glycogen changes in the vital organs of juvenile monkeys recovering from circulatory stasis.

c) The glycogen levels of the brain and other tissues of monkeys recovering from severe hypoglycemia (<20 mg% of blood glucose for a period of 6 hours).

d) The glycogen level of hypoxic newborn monkeys.

e) The effects of prolonged barbiturate anesthesia on the glycogen concentration of monkey tissues.

f) The effects of starvation on the cerebral glycogen concentration of monkeys.

Methods Employed: The deprivation of energy to the brain of the monkey was accomplished in a variety of ways: 1) anoxia (total asphyxia and circulatory stasis), 2) hypoxia, 3) hypoglycemia, 4) prolonged deep anesthesia, 5) starvation.

The brains were excised from anesthetized animals and frozen instantly at the temperature of liquid nitrogen in a mixture of isohexane:isopentane. The heart, lung, liver, spleen, kidney, and muscle (adductor) were also removed and frozen. The glycogen concentrations of these tissues were determined using modified classical methods.

Major Findings: The privation of energy to the brain resulted in an excessive accumulation of cerebral glycogen during the subsequent recovery period. This has been found not only for anoxia, deep anesthesia and hypoglycemia but also for starvation where the amount of cerebral glycogen accumulation was but half that obtained by other means of energy privation. The glycogen levels of the brain of the newborn and juvenile monkey responded in a similar manner to anoxic conditions.

Further work with anoxic newborn monkeys revealed that a minimum of 9 minutes of asphyxia were necessary to elicit the glycogen hyper-deposition response. This response once elicited was maximal and of the same magnitude for asphyxial episodes of from 9 to 30 minutes. Asphyxial episodes less than 9 minutes duration did not cause cerebral glycogen hyper-deposition. This glycogen response was antecedent to brain injury which occurred only after a minimum of 12 minutes of asphyxia.

The rate of cerebral glycogen synthesis during glycogen deposition was calculated to be 0.0064 mg/g/hr for starved monkeys, 0.033 mg/g/hr for anesthetized monkeys, 0.042 mg/g/hr for monkeys recovering from hypoglycemia, and 0.15 mg/g/hr for monkeys recovering from anoxia.

The cerebral glycogen response to starvation was not caused by blood sugar levels of these animals since these concentrations were normal. The post-anoxic hyper-deposition cerebral glycogen response were probably induced by the rapid initial depletion of the cerebral energy reserves. The same is true of the post hypoglycemic hyper-deposition response. In the latter case, however, the rate of glycogen synthesis had decreased probably due to the complete breakdown of the polysomes which are essential for protein synthesis.

Prolonged hypoxia did not affect the cerebral glycogen stores until the blood pH of the subject remained below 7.0 for 3 or more hours. Extended periods of such severe hypoxia caused extensive depletion of the cerebral glycogen stores (also observed in anoxia and hypoglycemia).

The liver glycogen stores were affected only by starvation and recovered rapidly after feeding was resumed. The evidence suggests the possibility that the condition of the liver could affect the cerebral glycogen stores during the early periods of starvation.

The glycogen of the heart and kidney were affected only during the anoxic periods. The glycogen levels of these organs returned to normal levels very quickly during the recovery periods, and showed no lasting effects of the anoxia. The glycogen of the muscle of the juvenile but not of the newborn monkey showed some detrimental effects on the fourth day of the recovery from anoxia. These organs were not affected by starvation or hypoglycemia.

The glycogen stores of the lungs and spleen were not affected by any of the experimental procedures.

Significance: The privation of energy to the brain by whatever the means eventually resulted in a decrease in neuronal activity which in turn was reflected by an accumulation of cerebral glycogen above control amounts. The cerebral glycogen accumulation was especially evident during the recovery periods following severe acute privation of oxygen or glucose and during periods of prolonged anesthesia. The periods of neuronal inactivity which followed the anoxia or hypoglycemia were sometimes evident by reversible neurological clinical symptoms experienced by some of these animals (such as blindness or partial paralysis). Animals surviving circulatory arrest or hypoglycemia exhibited high cerebral glycogen levels for 48 to 60 hours after the end of the assault.

The data also illustrates that two vastly different modes of energy privation (asphyxia and hypoglycemia) result in very similar biochemical responses indicating the possibility that the same reaction mechanisms are involved.

Experiments on the effects of starvation on cerebral glycogen suggest the possibility that the metabolic state of the liver (or other visceral organs) might affect these levels.

The data accumulated to date shows that the brain of the newborn and juvenile have the same sensitivity to anoxia although this is not true for the kidney or muscle tissues.

Proposed Course of Project: It is proposed to continue to try to quantify the relationship between cerebral glycogen and the activity of the nervous system. It is also proposed to find alternate means to reduce the metabolic activity of the brain to determine the relationship between the cerebral glycogen and the activity of the nervous system. Continue to look for chemical parameters which would yield information about the extent to which a noxious stimulus could cause brain damage.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LPP 1699
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Functions of forebrain commissures in learning

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Kazuo Hara, Ph.D.
Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: Department of Psychology, International Christian University, Mitaka, Tokyo, Japan

Man Years:

Total:	1.53
Professional:	.48
Other:	1.05

Project Description:

Objectives: To determine the role of the forebrain commissures in interhemispheric transfer of learning. Specific questions addressed are: the extent of transfer deficits in relation to the difficulty of the discrimination problems used in testing (Exp. I); timing of commissurotomy in relation to progress of learning (Exp. II); and degree of early sensory deprivation (Exp. III).

Methods Employed: Exp. I. Midsaggital sectioning of optic chiasma, corpus callosum and anterior commissures: learning of visual discrimination by one eye, interocular transfer testing, reversal learning and retesting for transfer with the opposite eye. Five different discriminanda are studied--brightness, color, pattern, form, and scene. Exp. II. Same as in Exp. I except that discriminations utilizing a variety of stimuli are presented in random order. Sectioning of corpus callosum and anterior commissure is postponed until completion of learning of third problem. Exp. III. Infant monkeys are subjected to midline sectioning of optic chiasma, anterior commissure and corpus callosum. At the same time, the eyelids of one eye are sutured closed. Subjects are raised utilizing only monocular vision for twelve months. The closed eye is then reopened and testing procedure as employed in Exp. I is followed.

Major Findings: Exp. I. Monkeys with total section of forebrain commissures and optic chiasma exhibited moderate degrees of interocular transfer of brightness, color, and scene, while no such transfer of pattern and form was observed. Those stimuli which manifested transfers are available in the natural habitat and thus have become components of social signaling which seems to be handled differently than neutral signals by the visual mechanism.

Exp. II. In contrast with evidence obtained with respect to tactual learning, sectioning the forebrain commissures after learning through one eye is followed by considerable decrease in transfer capability.

Exp. III. Study not completed.

Significance: Although lack of interocular transfer of visual learning after midline section of commissural fibers has been observed by others, ambiguities as to the nature of these deficits still remain. The present study clarifies some of the issues relating to discrimination difficulty, sophistication of subjects, and interproblem generalization of learning tasks. Also the last experiment hopes to provide critical tests determining the time development of visual perception and visual learning and of commissural functions relative to these processes. These studies bear upon our understanding of rehabilitation of human blindness (amblyopia ex anopsia) as well as for understanding the implications of sensory deprivation.

Proposed Course of Project: Complete Exp. III.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LPP 2020
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pathophysiology of brain injury produced by cardiovascular and pulmonary disorders

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators:
William A. Blank, M.D.
Francis W. Gamache, M.D.
Howard S. Kershner, M.D.
Gary S. Kopf, M.D.
Michael J. Labance, M.D.
David M. Mirvis, M.D.
Dennis J. Selkoe, M.D.
Harvey M. Shapiro, M.D.

Cooperating Units: None

Man Years:

Total:	4.06
Professional:	3.14
Other:	.92

Project Description:

Objectives: To develop animal experimental models of human brain disease produced by a variety of cardiovascular and pulmonary disorders. These include studies characterized by interferences with oxygen availability to the tissues either due to cessation of circulation (circulatory arrest), hypotension, pulmonary disease, myocardial abnormalities, or intoxications with such agents as carbon monoxide or cyanide.

Methods Employed: Juvenile or adult monkeys are anesthetized with pentobarbital, one femoral artery and vein catheterized for the recording of blood pressure, heart rate, and for withdrawal of blood samples for analysis of acid-base and respiratory gas state. Electrocardiographic and electroencephalographic leads are placed and a variety of physiologic parameters monitored including cerebral impedance, intracranial pressure, electrolyte composition of blood and CSF, EEG, glucose content of blood and CSF, etc. The animals are subjected to one of the variety of insults described above and measures are taken of the physiologic and biochemical changes produced. The animals are resuscitated and observed both neurologically during survival and pathologically following sacrifice.

Major Findings: The neuropathology of carefully regulated episodes of circulatory arrest has been defined as one which affects structures in the brain stem. Hemispheral structures including the cerebral cortex, nemispheral white matter, and basal ganglia remain unaffected. These findings are contrary to those described in the human and the present work has made it clear that the human patterns of injury following cardiac arrest are due not to the stasis of blood flow but to hypoxia which is incurred during efforts at resuscitation.

The extent of blood pressure lowering which the monkey can sustain without evidence of brain injury is in the order of 30mm Hg arterial blood pressure. Animals subjected to blood pressure lower than this may die during the period of hypotension usually due to cardiac or rhythmias or failure; may die in the early hours following blood pressure restoration of cardiogenic shock; or may die during the first 12-48 hours with severe brain swelling and brain stem compression. Only a small proportion of the animals subjected to severe hypotension survive to show static, long-term lesions.

Up to 14 minutes of cardiac arrest can be sustained at normal body temperatures without evidence of brain injury. Thereafter, loci in the brain stem show injury--the more prolonged the stasis the more widespread the injury. After 20 minutes of stasis survival is rare due to development of cardiogenic shock.

Hypoglycemia is associated with minor alterations in respiratory gas and acid-base state but leads to significant hypotension and circulatory impairment. When hypoglycemic animals are artificially respired, the patterns of pathology observed affect the basal ganglia, the cortex and the hippocampus. The long term changes consist of convolutional atrophy and dementia. Hypoglycemia does not seem, by itself, to lead to brain swelling.

Significance: These studies attempting to elucidate the pathophysiology and the pathogenesis of important patterns of brain injury in the human, caused by cardiovascular or pulmonary diseases, are important to pursue in order to develop techniques for their prevention or amelioration.

Proposed Course of Project: These studies will be continued to attempt further clarification of the pathophysiology of these various patterns of pathology caused by respiratory and circulatory disorders.

Honors and Awards: None

Publications:

Myers, R. E.: Two classes of dysergic brain abnormality and their conditions of occurrence. Arch. Neurol. 29: 394-399, 1973.

Ginsberg, M. D., and Myers, R. E.: Experimental carbon monoxide encephalopathy in the primate. I. Physiological and metabolic aspects. Arch. Neurol. 30: 202-208, 1974.

Ginsberg, M. D., and Myers, R. E.: Experimental carbon monoxide encephalopathy in the primate. II. Clinical aspects, neuropathology, and physiological correlation. Arch. Neurol. 30: 209-216, 1974.

Serial No. NDS(I)-73 LPP 2021
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Short- and long-term behavioral deficits produced by dysergic brain disease

Previous Serial Number: Same

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: Shun-ichi Yamaguchi, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.65
Professional:	.48
Other:	1.17

Project Description:

Objectives: To determine the short- and long-term effects on perceptual learning and memory function of a variety of brain insults produced by cardiovascular or pulmonary diseases and/or states of intoxication.

Methods Employed: Juvenile rhesus monkeys are trained on visual pattern discrimination tasks to choose one and to avoid the other of two visual stimuli. Banana pellet food reward is used to reinforce the correct pattern of responding. The monkeys are then subjected to episodes of energy deprivation (circulatory arrest, severe hypotension, severe hypoxia, total asphyxia, carbon monoxide intoxication, etc.). The animals are then resuscitated and again tested on the behavioral response. If response deficits are defined, the time course of restoration of performance is then determined. Permanent deficits are defined and quantitated.

Major Findings: Up to 14 minutes of circulatory arrest can be sustained with no effects on visual memory or discrimination.

Significance: The neurologic assessment of the clinical state of the rhesus monkey is difficult and leads only to crude measures of neurologic state. Utilization of more sophisticated behavioral test methods permit the assessment of subtle changes in overall nervous system activity, both with respect to temporary reversible disturbances and also to permanent deficits related to specific lesions.

Proposed Course of Project: To extend these test methods to assessment of the consequences of other pathologic states.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-73 LPP 2022
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neural mechanisms underlying social behavior and emotion in the rhesus monkey

Previous Serial Number: Same

Principal Investigator: Shun-ichi Yamaguchi, Ph.D.

Other Investigators: Ronald E. Myers, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.88
Professional:	.48
Other:	1.40

Project Description:

Objectives: To investigate the neural mechanisms underlying social behavior and emotion in rhesus monkey.

Methods Employed: A variety of techniques are used to study social behavior and emotion in the sub-human primate. In some instances, monkeys are placed in observation cages and their spontaneous patterns of vocalization are quantitated. In other instances, the general cage behavior of the animals and their reactions to challenge by the experimentor are defined. In other instances, the animals are placed in gang cages as small social groups and their social interactions determined.

Major Findings: Lesions of cortical areas related to speech in humans failed to alter the vocalization patterns of the rhesus monkey. On the other hand, areas of cortex having to do with the regulation and control of social behavior, including the prefrontal and anterior temporal cortex, produce major decreases or total abolition of vocal response. Bilateral lesions of subcortical structures revealed that amygdala and tegmentum of midbrain, both centers important to the regulation of social behavior, also alter the patterns of vocal response. Gang cage studies have demonstrated major deficits in all aspects of social behavior with lesions of prefrontal and anterior temporal cortex. Cingulate cortex lesions, on the other hand, produced only equivocal changes. Other studies with split-brain animals (destroyed crossing retinal fibers at optic chiasma) have shown that unilateral prefrontal and/or anterior temporal cortex lesions produce bilateral deficits as tested over the two sides of the body and in the two half-fields greater in the

half-field contralateral to the hemisphere of lesion. When corresponding lesions are added to second hemisphere, additional bilateral defects are demonstrable. Thus, elements exist both for laterality and bilaterality of these functions with respect to their cortical localization.

Significance: These studies which attempt to identify those brain structures involved in the regulation and control of social behavior are of major importance in our efforts to understand the biologic foundations of violence and other disorders of behavior.

Proposed Course of Project: Further examination of brain stem structures for their contribution to expression of social behavior and affect.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-74 LPP 2081
1. Laboratory of Perinatal Physiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental production of cerebral malformations by use of teratogenic agents or severe asphyxia during pregnancy

Previous Serial Number: None

Principal Investigator: Ronald E. Myers, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.94
Professional:	.14
Other:	.80

Project Description:

Objectives: To produce cerebral malformations in the developing rhesus monkey and sheep fetus by treating the mother with teratogenic agents or subjecting her to severe asphyxia at different stages of pregnancy. To carefully study the morbid anatomy of the cerebral malformations induced and to relate them to the time of pregnancy during which the insulting agent was administered. The patterns of induced morphologic abnormalities in the experimental animal will be compared with the known patterns of cerebral malformation in the human.

Methods Employed: Pregnant rhesus monkeys and sheep whose pregnancies length are known by virtue of dating of conception, are injected with MAM-acetate (methylazoxymethonal acetate, 10 mg/kg). Alternatively, the mothers are subjected to a single episode of severe asphyxia when the fetus is of different gestational ages. Near term, the mothers are anesthetized and the fetuses delivered. The body and organ weights of the newborn are determined and the brain carefully removed for gross and microscopic examination. The patterns of malformation induced are related to the time of gestation during which the insulting agent acted and the pathogenesis of the patterns of cerebral malformation are determined in relation to the development of anlaga tissue at the time of insult. The induced cerebral malformations are compared and contrasted with known patterns of malformation in the human.

Major Findings: The toxic dose of MAM-acetate has been defined for rhesus monkey and the first monkeys are being injected currently. Similarly, the severity of asphyxia required to produce brain injury of the developing fetus has been defined and efforts will be made to subject fetuses to severe asphyxia during earlier gestational ages.

Significance: The definition of the timing and of the inciting agents leading to cerebral malformations in the human is poorly understood. Thus, the above study, carefully carried out, should give major insights into the pathogenesis and morphogenesis of cerebral malformations as they occur in the human.

Proposed Course of Project: Injection of animals, harvesting of fetuses, weighing of internal organs, and examination of brains. Correlation with human findings.

Honors and Awards: None

Publications: None

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Stroke
Intramural Research

Ernst Freese, Chief

The Laboratory has investigated the effects of mutation or exposure to extracellular compounds on cellular membrane transport and differentiation. It has also analyzed the structure and replication of a mammalian virus. All investigations employed microorganisms or mammalian tissue cultures, because it was important for biochemical studies to have large numbers of identical cells. The use of microorganisms has the advantage that they grow rapidly and allow the isolation and genetic purification of many different mutants with desired properties. Thus a much more thorough examination of a problem is possible than would be feasible for the study of a similar phenomenon in higher organisms. Many biochemical reactions of microorganisms are identical or similar in higher organisms, so that the experimental findings of properly selected problems are significant for all organisms. Mammalian tissue cultures grow more slowly and do not permit the facile genetic manipulation of microorganisms, but their properties are closer to those of higher organisms and they allow the examination of problems that are particular to mammalian systems. The findings of the Laboratory are in the following.

1. Bacterial membrane transport and inhibition by lipophilic acids. The analysis of membrane transport in bacteria has been greatly advanced by the isolation of membrane vesicles which are essentially free of cytoplasm. Such vesicles can actively transport amino or keto acids if an external energy source such as NADH or glycerol-phosphate is provided. It has been shown that this transport is coupled to the electron transport system of B. subtilis and does not require ATP. The energy sources have to enter the vesicle before they can, by reaction with flavoproteins and the electron transport system, provide the energy for transport. Open or inverted vesicles oxidize NADH much more rapidly, without energizing transport, than closed vesicles. The transport into bacteria or its membrane vesicles is inhibited by all lipophilic acids such as fatty acids or parabens, which include most antimicrobial food additives, salicylates, and a number of antiseptics. In collaboration with the Department of Microbiology, Georgetown University, it was shown that the higher the lipophilic character (partition coefficient), the lower is the concentration needed to inhibit. This can be expressed quantitatively and thus has predictive value for the potential usefulness and hazards of lipophilic acids.

The transport of amino acids does not depend on the presence of sodium or potassium ions but it probably depends on a proton gradient which can be destroyed by the lipophilic acids. In order to characterize the molecular properties of transport systems more thoroughly, a mutant of B. subtilis was isolated which is resistant to hydroxy-aspartate; it is unable to transport this compound, aspartate, or glutamate into the cell, while the transport of other amino acids is not affected. Membrane vesicles of this mutant do not take up glutamate, but a significant uptake can be restored by addition of an extract of normal cell vesicles, which apparently contains the carrier protein for membrane transport. Experiments are now under way to isolate and characterize this factor. In this connection it was useful that B. subtilis in contrast to E. coli, does not seem to contain any periplasmic amino acid binding proteins.

2. Effect of lipophilic acids on human cells. Since lipophilic acids affect bacterial cell membranes, it was concluded that they should affect all cellular membranes, including those of human cells. In fact, it was observed, in collaboration with the Department of Microbiology, Georgetown University, that the growth of human cell lines, in particular HeLa cells, is inhibited by the same (or lower) concentrations of fatty acids, parabens, or salicylates that inhibit B. subtilis. Again, the concentration needed to inhibit decreases with increasing partition coefficient of the compound. In contrast to bacteria, the lipophilic acids do not inhibit the amino acid uptake into mammalian cells, apparently because this uptake is not energized by the electron transport system but at least for some amino acids by a sodium ion gradient. The observations show that lipophilic acids are not as innocuous to humans as one might conclude from the extensive and seemingly harmless use of antimicrobial food additives or aspirin. Actually, Dr. Feingold of the Kaiser-Permanente Medical Center has indications that food additives and salicylates greatly contribute to the problems of hyperactive children with minimal brain dysfunction; aspirin is known to irritate ulcers, and both aspirin and the antiseptic hexachlorophene can produce teratogenic effects. It is therefore clear that these compounds should not be used in higher amounts than absolutely necessary for thier antiseptic or drug effect.

Fatty acids with low molecular weight, in particular propionate and butyrate, inhibit the growth of HeLa cells at concentrations at which they are not yet effective against bacteria and they produce a remarkable change in the morphology of HeLa cells. The ordinarily rhomboid or round cells develop extremely long processes, so that the cells look like fibroblasts or serum-starved neuroblastoma cells 12

hours after butyrate addition or later. In collaboration with the Developmental and Metabolic Neurology Branch, NINDS, it was shown that butyrate causes an increase in the cellular sialyl-lactosyl ceramide content of the cell membrane by elevating the CMP-sialic acid: Lactosyl-ceramide-sialyltransferase activity 7-24 fold. Induction of this specific sialyltransferase is blocked by cycloheximide or actinomycin-D, indicating that its formation requires messenger RNA and protein synthesis. Since in vivo resting cells generally have long protrusions while cells growing in tissue cultures are transformed and behave similarly to cancer cells, butyrate seems to induce the reversion of cancer-like cell properties to a more normal resting cell type. The amount of cyclic AMP in these cells did not change under these conditions. Experiments to further correlate morphological changes with alterations in the glycosphingolipid content are under way. It appears possible that some of the lipophilic acids may become useful in the treatment of neoplastic cell growth.

3. Metabolic control of differentiation. Morphological cell differentiation generally does not start as long as cells are multiplying. The same is also observed in microorganisms in which differentiation can be observed by the production of heat resistant spores. The onset of differentiation can be further delayed by the addition of rapidly metabolizable carbon sources to the medium. Apparently, some compound derived from such carbon sources suppresses differentiation. In order to characterize a compound responsible for this suppression a number of mutants of B. subtilis have been isolated in which phosphorylated compounds can be formed from extracellular carbohydrates but cannot be metabolized into more than a few other compounds. The most restricted mutant is one which accumulates glucose-6-phosphate but is blocked in all three enzymes that normally metabolize this compound. Other mutants accumulate fructose-6-phosphate and L-α-glycerol-phosphate. The accumulation of any one of these phosphates prevents the formation of asymmetric cell septa, which represent the first step of bacillus differentiation that normally leads to the production of heat-resistant spores. When the accumulated phosphate has leaked out of the cell, differentiation can start, but the cell has by then changed its properties and develops abnormally as observed in the electron microscope. Since these phosphates are structurally quite dissimilar, it is likely that the accumulation of any organic phosphate suppresses differentiation. The detrimental effect of galactose-1-phosphate accumulation is well known in the disease of galactosemia; it can be expected that genetic defects resulting in the accumulation of other phosphates will also produce hereditary diseases or, more drastically, cause the abortion of the fetus in utero. As a by-product of these studies a mutant was isolated in which glucose-6-phosphate can only be converted to glucose-1-phosphate and on to the glucose or galactose-containing polymers.

The mutant exhibits at the end of growth extensive cell lysis which is caused by the accumulation of glucose-1-phosphate; this compound inhibits one of the two enzymes that convert glucosamine-6-phosphate to N-acetyl-glucosamine-1-phosphate and thus prevents sufficient cell wall synthesis. Consequently, the extent of mucopeptide synthesis is controlled by the rate at which glucose-1-phosphate is made and converted to glucose containing polymers.

Differentiation requires also the proper supply of various nutrients. When auxotrophic mutants are grown under conditions of limiting supply of the required nutrient, three responses of differentiation can be observed. 1) Some compounds such as leucine are needed in the medium only during growth and are provided during differentiation in sufficient amount by protein turnover. 2) Other compounds have to be present also during differentiation and some of them (adenine, nicotinic acid, riboflavin, thiamine, and tryptophan) are needed in much higher concentration (10 times or more) for optimal differentiation than for maximal growth. Similar observations have been made by other laboratories in mammals, where a teratogenic effect results when some vitamins are fed only in amounts sufficient for normal adult life. Microorganisms can be used to determine the reason for the high requirement of such compounds. For example, the concentration of thiamine required to allow optimal sporulation of B. subtilis can be reduced when the medium also contains acetate, apparently because pyruvate dehydrogenase is particularly important for differentiation. This has been verified by a pyruvate dehydrogenase mutant that can sporulate normally only when it is provided with 70 mM acetate. 3) A third type of auxotrophic mutant requires the presence of a small concentration of the missing compound during differentiation. For example, a glycerol-requiring mutant cannot sporulate when glycerol is added just once at the beginning of growth. The cells grow until glycerol has been used up and then cannot develop the special membranes required for differentiation. If more glycerol is added, the cells merely grow to a higher titer and then are faced with the same problem. Sporulation cannot start as long as glycerol is present, because that is rapidly metabolized and produces a suppressor of sporulation. A simple method was discovered, to supply the very small amount of glycerol-phosphate needed during differentiation: glycerol-phosphate itself is effective if it is added to the cells at a high concentration once at the beginning of growth. For glycerol-phosphate is taken up extremely slowly by B. subtilis and can enter the cells in just sufficient amounts to allow sporulation (septation) but not to suppress its onset. Although differentiation of other mutants isolated by the Laboratory could be partially restored previously, the restoration of completely normal sporulation in the glycerol-requiring mutant is remarkable; it suggests the possibility of restoring normal development or the maintenance of normal adult life also in some hereditary diseases.

A typical phenomenon of differentiation is that of commitment. It can also be observed with respect to microbial differentiation. Once sporulation has started, bacilli are committed to continue the development in spite of the addition of compounds which would ordinarily allow growth. Careful examinations of Bacillus megaterium have shown that the timing of this commitment is different with respect to different compounds. With respect to glutamate, the commitment occurs earlier than with respect to glucose; in both cases it is correlated with a loss of the cellular ability to take up these compounds. With respect to a rich medium, commitment occurs at a much later time. In that case, the prespore membrane apparently has to engulf the small sporulating cell almost completely before addition of these compounds can no longer reverse the sporulation development. The engulfed cell compartment is surrounded by two cell membranes which transport in opposite direction, hence no active transport is possible. Thus our findings indicate that the phenomenon of commitment is determined to a large extent by changes in the transport properties of cell membranes.

4. Control of mammalian virus replication. Myxo-, paramyxo, rhabdoviruses form a family of closely-related enveloped animal viruses that contain single-stranded RNA genomes. Although this group is responsible for many serious diseases such as influenza, rabies, mumps, and measles, very little is known about the virus replication. It is clear that the mode of replication is different from that of more thoroughly-investigated viruses, because the virus genome itself does not have messenger RNA properties but has to be transcribed into messenger RNA before it can function. Consequently, the specific RNA polymerase is carried by the virus itself, and viral RNA alone is unable to infect cells. The work done in the Laboratory concentrates so far on vesicular stomatitis virus. A number of other proteins have been found in this virus. Some of them (AG and M protein) are associated with the viral envelope. The enzymes protein kinase and a nucleoside diphosphokinase have been found; the core protein associated with RNA and the M protein can be specifically phosphorylated by protein kinase. The RNA polymerase of the virus can copy the ribonucleoprotein of the virus in vitro. At least one-third of the synthesized RNA contains polyadenylate (polyA) tracts. These RNA's can be separated by binding to oligo-dT-cellulose. The RNA polymerase is distinct from all RNA polymerases of the whole cell and thus represents a specific target for chemotherapeutic agents of a number of compounds investigated; tylorone inhibits RNA polymerase quite specifically; unfortunately, it is also toxic to cells. Other compounds are being examined.

Vesicular stomatitis virus, like many other RNA viruses, produces defective viral particles when infections are carried out at high multiplicity. The defective particles are smaller and contain a reduced complement of RNA. They are unable to replicate by themselves but can replicate when cells are coinfected with a normal virus. In this joint

replication the defective particles are preferentially multiplied so that the progeny contains only few infectious viruses (autointerference). Using different defective particles, one can show that each represents a unique viral sequence which obviously was produced by a deletion of the genome. Nevertheless, each of them can produce the autointerference the mechanism of which is being examined. It is hoped that some defective virus particles, which can be easily purified by sucrose gradient centrifugation, can be used as therapeutic agents against virus infection even after its onset. But since some defective viruses may cause cancer, a thorough investigation of their cellular effects is essential, before they could be recommended for therapy.

Serial No. NDS(I)-65 LMB/DB 1244
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

Individual Project Report
PHS-NIH
July 1, 1973 through June 30, 1974

Project Title: Control Mechanisms and Differentiation

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Peter H. Cooney, Ph.D., Anthony L. Rosner, Ph.D.

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.3
Other:	0.7

Project Description:

Objectives: The developmental processes leading to cellular differentiation begin when cells stop growing and end when a genetically-defined differentiated cell has been formed. Many essential regulatory phenomena involved in differentiation are undoubtedly similar in micro- and higher organisms. Since microorganisms can divide faster and large numbers of identical cells can be obtained for biochemical and genetic purposes, an analysis of essential processes involved in microbial differentiation should yield results relevant to all organisms faster than the direct investigation of complex mammalian systems. Auxotrophic and cacogenic (developmental) mutants can be isolated in microorganisms, and the arrest of the development can be statistically examined in many organisms by biochemical or cytological techniques. The results described below indicate that certain enzymes are particularly important to balance the production of organic phosphates, that some compounds are needed in much higher concentration for differentiation than for growth, and that the commitment of differentiation is partially due to alterations in membrane transport and partially to intracellular enzyme changes.

Methods Employed: Specific mutant strains were obtained by mutagenesis with γ-radiation, genetic transformation with DNA, and selection of the desired mutants in specific media or by resistance to phage SP8. A new

sporulation medium was developed (S3C) which contains vitamin-free casein hydrolysate and an amount of glycerol sufficient to prevent the cell lysis often observed at the end of growth. Commitment was followed in Bacillus megaterium by counting the number of phase-bright particles and the total number of particles under a phase-contrast microscope.

Major Findings: 1. Cell lysis of Bacillus subtilis caused by intracellular accumulation of glucose-1-phosphate. Mutants deficient in both glucose-6-phosphate dehydrogenase and phosphoglucoisomerase lyse 4-5 hours after growth in nutrient medium containing glucose or after prolonged incubation in the medium containing galactose. The lysis can be prevented by the addition of any other rapidly-metabolizeable carbon source such as fructose, glucosamine, or glycerol. The glucose-induced lysis is also abolished by introduction of a third mutation lacking phosphoglucomutase activity but not by a third mutation lacking UDP-glucose pyrophosphorylase or teichoic acid glucosyl transferase activity. Galactose-induced lysis is prevented only if the additional mutation abolished the UDP-glucose pyrophosphorylase activity. The results showed that lysis is caused by the intracellular accumulation of glucose-1-phosphate, which in turn inhibits at least one of the two enzymes that convert glucosamine-6-phosphate to N-acetyl glucosamine-1-phosphate. Thus the extent of mucopeptide synthesis is controlled by the rate at which glucose-1-phosphate can be converted into polysaccharides.

2. Suppression of differentiation by organic phosphates. A number of mutants have been isolated in which different organic phosphates accumulate because they cannot be converted into other cellular components. As long as such a compound accumulates inside the cell, differentiation is impossible, although the mutant can grow normally. When the carbon source giving rise to this accumulation has been used up and all the intracellular phosphate has leaked out of the cell, further development is possible but leads to abnormal differentiation; no spores are formed. Phosphates that have been isolated in this way are glucose-6-phosphate, fructose-6-phosphate, and L-α-glycerol phosphate. Since these three phosphates are structurally quite dissimilar, it is concluded that the accumulation of any organic phosphate leads to the abortion of differentiation.

3. Control of intracellular phosphate and alkaline phosphatase production. When Bacillus subtilis is grown in a medium containing phosphate, alkaline phosphatase remains repressed, whereas phosphate deficiency causes an enormous increase in the amount of this enzyme. In contrast, when cells enter the developmental period they produce at some time during this period an increased alkaline phosphatase activity in spite of the presence of inorganic phosphate in the medium. In order to determine whether the increase of alkaline phosphatase was due to a low concentration of intracellular phosphate in spite of a high concentration in the medium, the uptake and intracellular concentration of phosphates were measured. While the uptake of inorganic phosphate decreases and the concentration of acid-soluble organic

phosphate remains constant, the intracellular concentration of inorganic phosphate increases to about 30 mM, but only after the end of growth. Consequently, some compound other than inorganic phosphate must be responsible for the repression of alkaline phosphatase. The increase of inorganic phosphate apparently occurs sufficiently late that the developmental processes have already started and the phosphate can no longer suppress differentiation, because the cells sporulate well. Other experiments have shown that addition of glucose delays both the alkaline phosphatase increase and sporulation by about the same time, indicating a close correlation between the two processes.

4. Higher requirement of some compounds for differentiation than for growth. A particular sporulation medium (S3C) was used in order to demonstrate the requirement of vitamins and other nutritional components for growth and sporulation. It was found that the amount of adenine, nicotinic acid, riboflavin, thiamine, or tryptophan, each added to the medium of the mutant requiring the particular compounds for growth, is much higher for optimal sporulation than for maximal growth. The concentration of thiamine needed for sporulation can be reduced by a factor of 10 when the medium also contains acetate (5 mM), indicating the importance of pyruvate dehydrogenase for differentiation. Acetyl-CoA or its extracellular precursor acetate are used during differentiation for both ATP regeneration and membrane synthesis. The requirements of higher amounts of vitamins and other compounds for differentiation than for growth resembles a similar situation in mammalian systems in which it is known that the optimal concentration of vitamins needed by the adult organism is much lower than that required during gestation. Our results show that a vitamin deficiency can be partially overcome by feeding sufficient amounts of other nutrients which otherwise would have to be produced by the enzymes for which the cofactors are derived from the vitamins.

5. Control of commitment. Commitment to sporulation was examined with respect to dilution into fresh media and addition of specific sugars and amino acids. The earliest commitment during development occurred with respect to aspartate or glutamate addition. Dilution in fresh minimal sucrose medium or addition of glucose or fructose showed commitment at some intermediate time, while dilution in nutrient sporulation medium (supplemented with casein hydrolysate) exhibited commitment only at the time at which prespore septa had been made and had partially engulfed the small cell compartment. Reduced initial rates of uptake of sucrose, glucose, aspartate, and glutamate were seen to parallel the onset of commitment. Similar changes in uptake rates were not observed in the absence of sporulation. Use of chloramphenicol demonstrated that reductions in the rates of uptake of sucrose and glutamate depend on continued protein synthesis. In all cases commitment seems to involve changes in the cell membrane, for some compounds by decreasing transport systems and a final commitment by the removal of the prespore cell from contact with the medium via membrane engulfment.

Significance to Biomedical Research and Program of the Institute: The accumulation of some organic phosphates is responsible for hereditary diseases such as galactosemia may also cause teratogenic effects. Our results indicate that the accumulation of any organic phosphate interferes with differentiation. This indicates that the phosphate or acid moiety may interfere with some cellular reaction that is essential for differentiation but not for growth. Since this interference prevents the normal septation of bacilli, it shows that it affects membrane growth to a significant degree. Since in higher organisms the accumulation of organic phosphates also has neurological effects, again an effect on membrane growth or differentiation is indicated. Also, the alterations of cellular transport during commitment indicate the importance of membranes during differentiation.

Proposed Course of Project: By a correlation of electron microscopy and sporulation, the stage of membrane development will be determined at which cells are completely committed to continue their differentiation process. This could occur at the stage at which there is only one cell membrane surrounding the prespore cell or at a later stage at which there are already two such membranes, so that no small molecular weight compounds could be actively transported through both membranes. In addition, the processes will be examined which produce septation in the middle of the cell but at one end of the cell during sporulation. This transition indicates that the site of membrane septation can be controlled by a so far unknown process.

Honors and Awards: E. Freese was Honorary Chairman of the Sixth Annual Rochester International Conference on Environmental Toxicity, June 1973.

Publications:

Freese, E.: Defective sporulation and its restoration in auxotrophic and in cacogenic mutants of Bacillus subtilis. C.N.R.S. 227: 69-74, 1973.

Ichikawa, T. and Freese, E.: Alkaline phosphatase production of Bacillus subtilis. Biochim. Biophys. Acta 338: 473-479, 1974.

Speck, E. L. and Freese, E.: Control of metabolite secretion in Bacillus subtilis. J. Gen. Microbiol. 78: 261-275, 1973.

Freese, E.: Genetic effects of mutagens and of agents present in the human environment. In Sixth Annual Rochester International Conference on Environmental Toxicity, June 4-6, 1973, in press.

Prasad, C. and Freese, E.: Cell lysis of Bacillus subtilis caused by intracellular accumulation of glucose-1-phosphate. J. Bacteriol., in press.

Serial No. NDS(I)-71 LMB/DB 1886
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Developmental Cytology

Previous Serial Number: SAME

Principal Investigator: Elisabeth B. Freese, Ph.D.

Other Investigators: Yong K. Oh, Ph.D., Tsutomu Nishihara, Ph.D. (Visiting Fellow)

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	2.8
Other:	0.7

Project Description

Objectives: Differentiation consists of a sequential development leading from vegetatively-growing cells to highly differentiated ones. Some fundamental biochemical reactions are required for the process of differentiation but not for growth. We wanted to determine the effect of mutation in some of these important pathways and correlate changes of the morphological fine structure of cells with the enzyme alterations. We used for this purpose the sporulating cells of Bacillus subtilis in which mutants can be easily isolated and the mutations can be genetically transformed into other cells. In particular, mutants accumulating organic phosphates, lacking the ability to produce vitamins and nucleic acid bases, or requiring glycerol, were investigated.

Methods Employed: The mutants were derived from the transformable 168 strain by γ-irradiation. They were transformed into other cells in order to insure the presence of only a single mutated gene. The amount of the metabolism of glycerol was followed by means of ^{14}C (UL)-glycerol. Cells were collected on membrane filters, washed with 5 ml 0.1 M potassium-phosphate, pH 7, and 1 mM magnesium chloride and extracted with 1/50 concentrated formic acid. Both extracted media were chromatographed on Whatman #1 paper in descending isopropanol/acetic acid/water (3:1:1); the R/f's of glycerol and glycerol-P are 0.7 and 0.35 respectively.

For the morphological analysis ultra-thin sections were studied with the electron microscope, EM 6B of AEI. The mutants were grown for 6 and occasionally 24 hours after the end of exponential growth, centrifuged, fixed by glutaraldehyde, stained with osmium tetroxide and uranyl acetate, dehydrated, embedded in Epon, and after sectioning with the Reichert microtome OmU2, stained by lead citrate.

Major Findings: 1. Developmental properties of glycerol-requiring mutants. A glycerol-requiring mutant (61106) derived from B. subtilis 168 grows in nutrient sporulation medium that contains 100 mM additional potassium-phosphate to a lower A_{600} (1.1) than without the phosphate addition (A_{600} = 2). At the end of growth it releases ATP into the phosphate medium, whereas it runs out of ATP in the medium without the added phosphate. Under the electron microscope a severe collapse of the cytoplasmic membrane, separated from the cell wall, is seen in the phosphate medium, while the collapse is much less severe without phosphate. These effects may result from the accumulation of free fatty acids, which was observed toward the end of growth in both media but earlier in the phosphate medium. In both media cells sporulate poorly (frequency 10^{-3}). This inability to sporulate could not be restored by the addition of more glycerol because growth merely proceeded to a higher A_{600} until all glycerol had been used up, but sporulation still remained low. The cells are obviously in a severe dilemma because as long as they have glycerol available they can grow, but the catabolites of glycerol suppress sporulation; as soon as glycerol has been used up the cells could start to sporulate, but they need some glycerol-phosphate for the formation of prespore septa. Sporulation could be restored by the addition of 5 mM or more DL-α-glycerol-phosphate which is taken up extremely slowly by the cells but allows enough production of phospholipids to restore prespore septation and sporulation. The lack of sporulation is not due to a deficiency in cell wall teichoic acids because a glycerol-requiring mutant (61471) derived from B. subtilis W23, which produces cell wall polyribitol teichoic acid, could also not sporulate except when glycerol-phosphate was added. The results show that a much more precise balance of metabolites is required for sporulation than for growth.

2. Stage of development at which suppressed mutants are arrested. A large number of mutants has been isolated in this laboratory and used to show that the deficiency of a vitamin or a nucleic acid precursor or the excess of phosphorylated compounds suppresses sporulation. All these mutants have been investigated under the electron microscope 6 hours after the end of growth, and it was found that they are all unable to produce the asymmetric prespore septa which are a prerequisite for the sporulation process. It is therefore concluded that the accumulation of phosphorylated intermediates and perhaps any acids prevents cells from proceeding beyond a very early stage of development by interfering with some so far not-identified metabolic process.

3. Motility of B. subtilis during growth and sporulation. When the standard strain of Bacillus subtilis reaches the end of growth, the cells consistently loose their motility although they maintain a constant concentration of ATP and can later sporulate. To determine whether the ability to sporulate was correlated with the loss of motility, a number of sporulation mutants blocked at different times of development were statistically investigated under the microscope. In all mutants motility decreased after the end of growth; apparently no portion of the sporulation process is necessary for this decline. However, decrease of motility was not correlated to the loss of flagellae as was shown by negative staining in the electron microscope; apparently some metabolic reactions that occur at the end of growth inhibit the motility of cells.

Significance to Biomedical Research and the Program of the Institute. Whereas the work reported here was done in bacilli, the principles elucidated should be the same in higher organisms. In particular, the two enzymes by which glycerol-phosphate can be synthesized and catabolized are present in higher organisms and their role presumably is again to maintain an accurately-controlled balance of the amount of glycerol-phosphate. The finding that malate can prevent the glycerol-phosphate accumulation and that glycerol-phosphate can be used to cure a glycerol-requiring mutant indicates that one could use similar techniques to alleviate the effects of hereditary diseases in man. The observation that the normal development of the prespore membrane septum was prevented in all mutants indicates a strong effect of phosphate (or acid) accumulation on membrane synthesis which may explain why many human hereditary diseases also have neurological consequences.

Proposed Course of Project: The mechanism by which phosphorylated intermediates suppress differentiation will be investigated in the hope that this will also provide an explanation for the detrimental effect of phosphorylated intermediates that accumulate in human hereditary diseases. In neither case is the reason for the deleterious effect known. Electron microscopy will also be used to investigate the molecular structures (microtubules) that assemble in HeLa cells in the presence of butyrate and cause the appearance of long cellular processes which are usually observed only in fibroblasts.

Honors and Awards: None

Publications:

Freese, E. and Marks, C. L.: The developmental block in citric acid cycle mutants of Bacillus subtilis. J. Bacteriol. 116: 1466-1468, 1973.

Serial No. NDS(I)-72 LMB/DB 1962
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanism and Control of Membrane Transport

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Martin Diesterhaft, Ph.D., Michael L. Hampton, Ph.D., Barbara Levin, Ph.D. (Guest Worker)

Cooperating Units: None

Man Years

Total:	2.8
Professional:	2.5
Other:	0.3

Project Description

Objectives: Since it has become possible to isolate membrane vesicles from bacteria, one can study the transport of substrates through membranes independent of cytoplasmic components and compare it with that into whole cells. In this way it has been established that the transport of amino or keto acids is coupled to the electron transport system, but the presence or production of ATP is not required. The aim of the present work was to determine how transport can be energized by different energy sources, by which compounds it can be inhibited, and how cellular transport functions. It is anticipated that the findings can be employed to understand and improve the antimicrobial action of compounds important for Public Health and to minimize the potential harm to human development and nerve function. Compounds studied in particular are food additives, antiseptics (Hexachlorophene), and analgesics and antipyretics (aspirin).

Methods Employed: Different compounds were screened for their inhibition of growth and oxygen consumption. In this way the inhibiting concentration range has been established. Membrane vesicles were isolated by protoplasting the cells with lysozyme and lysing them by rapid dilution. By growing the cells in the presence of glycerol, glycerophosphate dehydrogenase was induced, allowing glycerol phosphate to be used as energy source.

Mutant strains were selected on the basis of their resistance to inhibitory levels of the hydroxy-aspartate and other aspartate or glutamate analogs. In this manner it was possible to select specific amino acid transport mutants. A rapid method for the production of large quantities of membrane vesicles has been perfected. The entire operation is carried out in dilute buffer in the presence of lysozyme; once the wall is dissolved, the resultant protoplast bursts and spews out its cytoplasm. The nucleic acids are then hydrolyzed with DNAase and RNAase, the membranes are harvested, washed, and stored.

Major Findings: 1. Mechanism by which NADH energizes transport into membrane vesicles. In membrane vesicles the amount of amino acid taken up per oxygen consumed is much higher with α-glycerophosphate than with NADH as energy source. This phenomenon results from the presence of open or inverted vesicles in the membrane preparation. Cells or protoplasts of Bacillus subtilis, preincubated to give very low endogenous energy production, oxidize 40 mM DL-α-glycerol phosphate 13 times faster than 20 mM NADH; cell breakage increases the rate of NADH oxidation dramatically, whereas the rate of glycerol phosphate oxidation changes only little. While the oxidation of glycerol phosphate shows one apparent K_m (6 mM), that of NADH exhibits two K_m's (0.2 and 2.8 mM). With whole cells, only the high K_m is observed. Also, the amino acid transport into membrane vesicles shows only the high K_m. Rotenone (0.4 mM) inhibits vesicular oxygen consumption much more than amino acid uptake, apparently because it cannot enter the vesicles. It is therefore concluded that NADH energizes amino acid uptake only from within the membrane vesicles. This explains the high K_m, because NADH penetrates the vesicular membrane only slowly. Glycerol phosphate also acts only from within, but in that case the rate-limiting reaction is that of glycerophosphate dehydrogenase.

2. Inhibition of growth, amino acid transport, and oxygen consumption by food additives and antiseptics. Most antimicrobial food additives and antiseptics now used are lipophilic acids; they inhibit growth of unwanted microorganisms. These compounds, as well as other so far not used lipophilic acids and long chain fatty acids, all inhibit the growth of Bacillus subtilis, its consumption of oxygen, and its uptake of amino acids. The inhibitory strength of these compounds can be compared by plotting an inhibition index against the logarithm of the inhibitor concentration. It was found that the inhibition increases with the lipophilic character of the compound and with decreasing pH. Apparently, the inhibition is caused by the attachment of the free acid to the cell membrane. The effect of hexachlorophene is presently examined in more detail, because it attaches apparently irreversibly to cell membranes and is effective in inhibiting growth at extremely small concentrations. It is also known to cause brain damage in children.

Whereas Na and K ions have a profound effect on transport, in some organisms no such effect was found in whole cells or in membrane vesicles of B. subtilis when they were used under a variety of conditions known to

stimulate transport in other systems. Thus the transport of these two amino acids in B. subtilis occurs in the absence of Na or K ion gradients. It probably requires a proton gradient which can be eliminated by the lipophilic acids.

3. Reconstitution of glutamate transport in mutant vesicles and binding protein assays. In order to biochemically characterize specific components of the transport system, it is necessary to define conditions under which these components can be resolved and analyzed separately. One such method utilizes a reconstitution assay. A preparation of membranes from the standard strain was treated with 0.5 mM guanodine HCl, the membranes were removed, and the supernatant was added to inactive vesicles of a mutant strain unable to transport glutamate. This mutant (60346) had been isolated by its ability to grow in the presence of the inhibitor hydroxyaspartate. After allowing sufficient time for reassembly, the membranes were centrifuged, washed, and assayed. Under these assay conditions it is possible in the mutant preparation to regain up to 30% of the glutamate transport activity of standard strain membranes. To further characterize such possible restoring activities the factor was assayed by its ability to bind radioactive glutamic acid. Two such assays have been used. The first, a Millipore filter binding assay, depends on the ability of the binding protein to adhere to the nitrocellulose filters. The second method utilizes equilibrium dialysis. The first assay has the advantages of ease of operation and immediacy of results. The second has the advantages of greater sensitivity and usefulness under a variety of experimental conditions. With these assays it has been possible to extract binding proteins from the membranes and to concentrate such preparations by ultrafiltration.

Significance to Biomedical Research and the Program of the Institute: Membrane transport is a universal phenomenon of all living cells and basic mechanisms of transport will likely be the same. The advantages offered by the use of a simple organism include the relative ease with which biochemical and genetic studies can be conducted. The only membrane transport protein so far isolated is that of Na-K-ATPase. The isolation of a carrier of substrate transport is therefore of general importance and our B. subtilis mutants offer unique advantages. The studies on transport inhibition are relevant to the teratogenicity of some antiseptics, the mechanism of these effects being unknown. Only when this mechanism is understood can safe antiseptics be selected, instead of the present haphazard method of using what has been only partially investigated.

Proposed Course of Project: The reconstitution and binding protein work is most difficult but bears the promise of being the most significant. Great effort is planned to be spent on this aspect of the investigation. The isolation and characterization of the transport mutants will continue to yield significant new information and will be pursued. The investigation of antiseptics will be pursued and the results will be compared with those obtained in higher organisms to determine which antiseptics are safe and why some of them are teratogenic.

Honors and Awards: None

Publications

Freese, E.: Thresholds in toxic, teratogenic, mutagenic and carcinogenic effects. Environ. Health Perspec., December 1973.

Sheu, C. W. and Freese, E.: Lipopolysaccharide layer protection of Gram-negative bacteria against inhibition by long chain fatty acids. J. Bacteriol. 115: 869-875, 1973.

Hampton, M. L. and Freese, E.: Explanation for the apparent inefficiency of NADH in energizing amino acid transport in membrane vesicles. J. Bacteriol., in press.

Yoshida, A. and Freese, E.: Lactate dehydrogenase from Bacillus subtilis. In Wood, W. A. (Ed.): Methods in Enzymology. New York, Academic Press, in press.

Serial No. NDS(I)-72 LMB/DB 1963
1. Lab. of Molecular Biology
2. Section on Developmental Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Genetic Regulation in Human Diploid Tissue Culture

Previous Serial Number: SAME

Principal Investigators: Jeffrey L. Simmons, M.D., and Ernst Freese, Ph.D.

Other Investigators: Richard Henneberry, Ph.D.

Cooperating Units: Developmental and Metabolic Neurology Branch, NINDS
Behavioral Biology Branch, NICHD

Man Years:

Total:	2.5
Professional:	2.2
Other:	0.3

Project Description:

Objectives: Work in this laboratory has continued to focus on compounds affecting the morphological expression of the genome, especially those alterations caused by lipophilic acids. This aspect of control has been of great interest because some of the compounds, including butyrate and the food additive propionate, which inhibit growth also produce the appearance of long cell protrusions, thereby reversing the shape changes caused by viral transformation. Whereas previous studies have indicated a primary role in these processes for butyryl cyclic AMP, it is clear from our recent work that simpler agents can produce even more profound morphological changes.

These studies should contribute to the understanding of cell shape control and might contribute to the elimination or reduction in the rate of neoplastic growth.

Methods Employed: 1. Morphological studies: Changes in cellular behavior resulting from an alteration of environment are often not adequately demonstrated with still phase micrographs because cells undergo normally various shapes. Therefore, we have examined the effect of the Na-butyrate on cells utilizing Normarski interference contrast optics and phase cine-microphotographic techniques. We have constructed a microenvironment cinemicroscope stage and have taken films for analysis of mitotic sequence

M-phase duration, synchronization, and process elongation.

2. Amino acid transport studies: Amino acid transport measurements are usually performed by artificially suspending the cells and exposing them to radioactive substrates for a defined period. A number of difficulties arise in this approach: a) the cells are removed from their normal physical environment; b) they must usually be exposed to some digestive compound (e.g., trypsin) to achieve adequate separation; c) rapid measurements to determine initial rate kinetics are often not feasible because of physical limitations. We have used a cover slip monolayer method which is remarkably flexible and reproducible. In addition, we have labeled the cells for long times with a tritiated compound in order to monitor the number of cells; the incorporation of a ^{14}C compound is then measured by evaluating each radioactive label separately in a scintillation counter. Cell volumes could be determined by comparing inulin and urea spaces.

3. Enzyme induction studies: The effect of Na-butyrate on induction of several enzymes in the pathway of ganglioside biosynthesis was studied utilizing labelled precursors. In addition, thin-layer chromatography was used to assay specific gangliosides.

Major Findings: 1. Effect of butyrate on cell growth and amino acid transport. Although butyrate strongly inhibits amino acid transport in bacterial systems, neither the diploid WI-38 nor the heteroploid HeLa cell lines are inhibited. ATP levels are also not altered. It is concluded that growth inhibition in HeLa cells does not result from inadequate intracellular amino acid or energy levels.

2. Effect of butyrate on morphology of HeLa cells. Cinemicrophotographic studies demonstrate that Na-butyrate is effective only during the G1 phase of the cell cycle. In addition, partial synchronization of the cell population is seen; the time course and details of process elongation are currently being analyzed.

3. Effect of butyrate on ganglioside biosynthetic pathway. Na-butyrate (and selected other lipophilic compounds) which alter the morphology of HeLa cells in culture have been shown to induce a specific sialyltransferase. This induction is inhibitable by cycloheximide and actinomycin D, and the enzyme level decreases again with the removal of butyrate. This enzyme induction has been well-correlated with the appearance of the typical butyrate-induced morphological alteration but not with the growth-inhibiting effect of other lipophilic compounds. The effect appears not to be cyclic AMP-mediated.

Significance to Biomedical Research and the Program of the Institute: The aspects of our work are particularly applicable to three significant areas of application. First, may provide a key to the understanding of morphological changes of cells in vivo. Our studies suggest an analogy between the elongation of processes by HeLa cells and the growth of axonal processes in fetal development and neuronal regeneration. By means of

butyrate the control of this process and its dependence of subcellular organization (microtubules, microfilaments, myosin, Golgi) can be examined. Second, these studies attempt to approach the general question of cellular regulation, including the balance between cyclic nucleotides and other cytoplasmic agents in controlling genomic expression. Third, the general issue of the effect of lipophilic agents, food additives, and antiseptics on human cells and the correlation to the teratogenic effect of some of these agents is under attack. Some of the examined agents may become useful in the treatment of neoplastic cell growth.

Proposed Course of Project. 1. Continued exploration of the mode of action of lipophilic agents, utilizing studies of fatty acid transport and binding as well as measurements of changes in subcellular cation equilibria. 2. Further investigation of mechanisms whereby butyrate and other compounds induce sialyltransferase I with special reference to parallelism to the role of cyclic AMP. 3. Studies on the role of microtubules and microfilaments in process elongation and growth, including mechanisms by which the pattern of that growth may be altered. 4. Examination of the effect of lipophilic acid on the growth of tumor cells in vitro and possibly in vivo.

Honors and Awards: None

Publications:

Henneberry, R. C. and Freese, E.: Temperature dependent permeability changes of Bacillus subtilis and incorporation of nucleotides into DNA. Biochem. Biophys. Res. Commun. 55: 788-797, 1973.

Serial No. NDS(I)-65 LMB/MV 1208
1. Lab. of Molecular Biology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ribonucleic Acid and the Regulation of Cellular Metabolism

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Gudrun Stamminger, Ph.D. (Guest Worker)

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Other:	0.

Project Description:

Objectives: The nucleic acid content of cells is subject to control by several regulatory mechanisms. The synthesis of certain species of messenger RNA is elicited by the presence or absence of specific metabolites in the cell milieu. The accumulation of ribosomal RNA, however, does not respond to the availability of specific nutrients but responds, rather, to the fitness of the medium for growth and the growth rate of the cell. Transfer RNA, on the other hand, responds neither to specific nutrients nor to the growth rate but is constant under most conditions of growth. The details of the mechanisms underlying these observations are at present obscure and are the subject of this project. We have concentrated on three aspects of these control mechanisms: 1) Control of ribosomal RNA synthesis in growing and nongrowing microbial cells. 2) The cell-free transcription of ribosomal RNA. 3) Control of ribosomal RNA during early embryogenesis.

Methods Employed: Standard biochemical techniques are employed for the purification of RNA, DNA and enzymes. Analysis of complex mixtures of RNA are performed by chromatography on Sephadex G-100 or methylated albumin or polyacrylamide gel electrophoresis. Messenger RNA is estimated by molecular hybridization with homologous DNA. The rates of synthesis of individual RNA species are determined from the kinetics of incorporation of radioactive precursors into the species.

Major Findings: With the discovery of altered catabolism of the guanosine tetraphosphate in certain mutant strains we initiated the final phase of this research project. These mutants, which can degrade ppGpp at only 1/20-1/40 the rate observed in wild-type strains were shown to result from a mutation at the spoT locus on the E. coli chromosome. These same mutants are unable to synthesize the guanosine pentaphosphate, pppGpp. These and other characteristics demonstrate that ppGpp is the precursor and not the product of the pentaphosphate, pppGpp, as many had assumed. Since under most circumstances these mutants regulate the ribosomal RNA appropriate-16, it is concluded that the tetraphosphate, not the pentaphosphate must be considered as the putative effector molecule. Even in this altered mutant the relationship between guanosine tetraphosphate and rRNA synthesis is maintained: when the intracellular ppGpp concentration is high, rRNA synthesis is inhibited. During the slow catabolism of the tetraphosphate, after the readdition of the deprived amino acid, there was a reciprocal increase in the rate of rRNA synthesis during the recovery period and full rRNA synthesis was not achieved until the ppGpp level fell to the basal value, 30 minutes later.

Significance to biomedical research and the program of the Institute: Controls of nucleic acid synthesis in bacterial systems represent examples of regulatory mechanisms which are more amenable to experimental investigation than those of the more slowly-growing mammalian systems. It is anticipated that information obtained from these simpler systems will help form a basis upon which an investigation of the more complex regulatory mechanisms of higher organisms can be built and eventually an understanding and control of disease in these systems can be obtained.

Proposed Course of Project: The work described here completes our analysis of the regulation of ribosomal RNA synthesis during normal growth and adverse physiological conditions. Our results, together with those of other laboratories, very strongly have implicated the guanosine tetraphosphate, ppGpp, as the controlling element in this regulation. The final link in this proof must come from cell-free experiments in which the in vitro synthesis of rRNA is shown to be specifically inhibited by the guanosine tetraphosphate and a specific repressor molecule. These experiments are already under way at Columbia University, Harvard University, and Copenhagen, and preliminary positive results have been reported. We are therefore terminating further work on this project.

Honors and Awards: Invited speaker at Gordon Conference on Biological Regulatory Mechanisms, July 1972. Invited to review the field for the series, "Protein Synthesis, a Series of Advances," Marcel Dekker, Inc. Invited to review the field for Cell, 1974.

Publications:

Gallant, J. and Lazzarini, R.A.: Regulation of RNA synthesis and degradation in microorganisms. In McConkey, E. (ed.): Protein Synthesis, a Series of Advances. New York, Marcel Dekker, 1974.

Stamminger, G. and Lazzarini, R. A.: Altered metabolism of the guanosine tetraphosphate, ppGpp, in mutants of E. coli. Cell 1: 85-90, 1974.

Serial No. NDS(I)-73 LMB/MV 2026
1. Lab. of Molecular Biology
2. Molecular Virology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of Viral Nucleic Acids Synthesis in Animal Cells

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Leslye Johnson, M.S., Sabih Khan, Ph.D. (Guest Worker), Michael Murphy, Ph.D., Gudrun Stamminger, Ph.D. (Guest Worker)

Cooperating Units: Laboratory of Biochemistry, NCI
Serial No. NCI/LB 379

Man Years

Total:	4.25
Professional:	4.00
Other:	.25

Project Description:

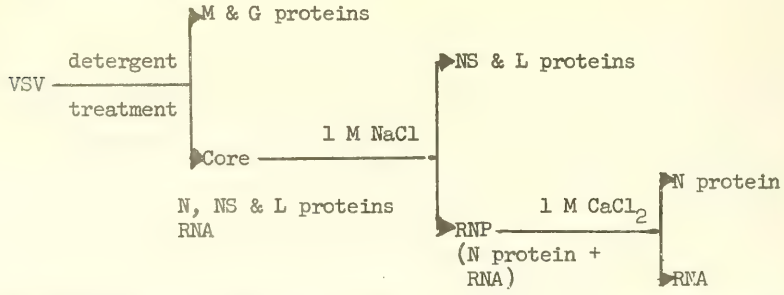
Objectives: The myxo-, paramyxo, and rhabdoviruses form a family of closely-related enveloped animal viruses which contain single-stranded RNA genomes. Although this group is responsible for many serious diseases, for example, influenza, rabies, mumps, and measles, very little is known about their replication. From the little that is known, it is clear that their mode of replication is different from that described for poliovirus, the RNA tumor viruses, or the RNA bacteriophages QB and MS2. The replication of the myxo-, paramyxo, rhabdoviruses is independent of host cell DNA function and takes place in the presence of actinomycin D. The single-stranded RNA viral genome is transcribed into a complementary RNA which serves as messenger RNA for the viral protein synthesis. This synthesis of complementary RNA is performed by an RNA-dependent RNA polymerase that is found in the virion itself. Later, in the infectious process, RNA of the same complementarity as the virion RNA is produced and packaged in the progeny virus particles. The details of the mechanism underlying the synthesis of RNA of both complementations senses and the regulation and coordination of these syntheses with each other are the subject of this project. Since the uninfected host

cell does not carry out an RNA-dependent RNA synthesis, these viral processes probably represent the most accessible and specific target for therapeutic agents.

Methods Employed: Viruses are concentrated and purified by ammonium sulphate precipitation, differential centrifugation, and isopycnic banding. Standard biochemical techniques are employed for the purification of RNA, DNA, and enzymes. Analysis of complex mixtures of RNA are performed by polyacrylamide gel electrophoresis or by chromatography on oligodeoxythymidylate cellulose columns. Complementarity of RNA is established by molecular duplex formation with purified virion RNA.

Major Findings: The work carried out on this project during the last year falls conveniently into three interrelated categories: structure-function relationships of the virion components, RNA synthesis in the infected cell, and autointerference. Each of these topics will be discussed in turn.

1. Structure-function relationships of virion components. VSV virions contain an RNA polymerase that can transcribe the single-stranded RNA genome of the virus outside of the infected cell. This synthesis is catalyzed by detergent-treated virus and by certain subviral components. The details, however, are completely obscure, and until recently it was not known which virion protein catalyzes transcription, nor was the nature of the template understood. We have undertaken a systematic dismantling of purified VSV virions in order to explore the structure-function relationship. The scheme employed is summarized schematically below:



The core obtained by detergent treatment contains all of the RNA polymerase activity in the original VSV virion. However, the ribonuclear protein obtained from the core by high salt treatment is inactive, as is the supernatant solution containing the proteins NS and L. Recombining the supernatant and RNP, however, regenerates full RNA polymerase activity. Work by others has demonstrated that the L protein is in fact the RNA polymerase. We have extended these studies and shown that the template is the ribonuclear protein

particle. Neither the purified virion RNA nor various synthetic homo- and hetero-ribonucleic acids can substitute for the RNP. Recently, we have succeeded in removing the N protein from the RNA, thus resolving the RNP into its two component parts. We are currently trying to reassemble RNP's, using both virion RNA and heterologous RNA's. These materials will be used in the study of the template requirements of the RNA polymerase.

In collaboration with Drs. Shelton and Ornstein (LB/NCI), we have visualized the structures of each intermediate during the dismantling by means of electron microscopy. Of particular interest is the conclusion drawn from this study that the RNP is not a long RNA chain encased in protein like a coaxial cable but rather a two-component ribbon in which the N protein is attached to only one side of the RNA throughout its length. This structure suggests a way in which the RNP could serve as the template for RNA synthesis, a process that undoubtedly involves base pairing of the incoming nucleotides to the template strands. The N protein is attached to the sugar phosphate backbone of the RNA, leaving the nucleoside bases fully exposed and accessible for bases pairing.

The RNA-dependent RNA polymerase of VSV virions represents a purely viral function for which the host cell has no counterpart. Consequently, this process probably represents the most accessible and specific target for chemotherapeutic agents. During the last year we have undertaken a search for such compounds, drawing on the collections of the National Cancer Institute and Merck, Inc. Of those tested, tylorone and its analogs seem the most promising.

2. RNA transcription in the infected cell. We have purified from infected cells an RNA-protein complex that behaves in every way like the core of the isolated virion. It sediments in the ultracentrifuge with a constant of 140S, it is composed of a virion RNA of 4×10^6 daltons and multiple copies of two proteins. This complex shows virtually all of the RNA polymerase activity of the infected cell. It can be further fractionated according to the scheme used for the virus. Furthermore, the RNA synthesized by the complex messenger RNA has polyadenylate tracks at its 3' termini, just like the product of the virus core catalyzed reaction. This coincidence of properties supports our conclusion that the study of RNA synthesis by purified VSV virions is relevant to the RNA synthesis that takes place in the host cell during the course of infection.

Viral RNA synthesized in the whole infected cell can be separated into three size classes of RNA-protein complexes: a 140S viral RNP containing viral RNA and N protein, an 80S complex containing an mRNA of 1.7×10^6 daltons, and a 60S complex containing an mRNA of 0.4×10^6 daltons. We have developed a fractionation procedure based on these sedimentation differences that yields virtually pure RNA of each class. By RNA-RNA annealing we have demonstrated that the two classes of mRNA are nonoverlapping classes,

i.e., genetic information present in one class of mRNA is not present in the other. This purification procedure of the nonoverlapping mRNA classes is important, because it yields two purified RNA classes that can be used to probe the mechanism of VSV replication. This application is illustrated below.

3. Autointerference. VSV, like many other RNA viruses, elaborates defective viral particles when infections are carried out at high multiplicity. These defective particles contain the viral proteins and lipids but less than a full complement of RNA. These particles are incapable of replication by themselves and can only replicate with the aid of a complete virus. However, in this joint replication, the defective particles are preferentially replicated, and the progeny contain relatively few infectious viruses. We are currently exploring the molecular basis for this interference. There are two common classes of defective particles for VSV: one containing approximately 40% of the normal complement of RNA, the other containing only 25% of the normal complement. Using the two classes of mRNA described above, we were able to describe three important properties of the autointerfering defective particles. They are: a. The RNA contained in the defective particle is virion RNA and not host RNA. b. The RNA from the defective particles is not a random assortment of viral RNA sequences of the appropriate length but rather represents specific regions of the viral genome. In effect, they are deletion mutants. c. The two classes of defective RNA's show no sequence homology, i.e., each RNA represents a distinct region of the genome of no overlap. This means that there is not a single genetic element needed for autointerference. If a particular sequence of RNA is necessary for autointerference, it must be represented in the VSV genome more than one time.

Significance to biomedical research and the program of the Institute: Viral diseases of the central nervous system (CNS) usually occur as a complication rather than a normal consequence of infection. Nevertheless, many members of the myxo-, paramyxo-, rhabdovirus family, either exceptionally or as a normal consequence, infect the CNS, causing encephalitis or meningitis. For example, the myxovirus, fowl plague virus, can cause a diffuse encephalitis. Examples within the paramyxovirus family are more numerous and include the viruses causing mumps, measles, canine distemper, and Newcastle's disease. Rabies virus is an outstanding example in the rhabdofamily. Despite their importance to medical neurology, very little is known about the regulation and mode of replication of these viruses in the host organism. The study of viral infections in cultured cells will reveal the molecular biological characteristics of the replication in a manner uncomplicated by the host response to the infection. It is anticipated that the study will delineate characteristics that can be exploited in containing and limiting viral infection to non-neural tissues or in the treatment or prevention of the viral infection.

Proposed Course of Project: 1) Further establish the physical map of VSV by RNA-RNA annealing experiments. This will entail determining the polarity and the sequence of genes on the genome. 2) Initiate investigations into the replication of VSV during the infectious process. 3) Examine the nature of the RNA isolated from certain classes of defective interfering particles.

Honors and Awards: Chairman of the session on Regulation of RNA Synthesis and Utilization--Gordon Conference on Biological Regulatory Mechanisms, 1974.

Publications: None

ANNUAL REPORT
July 1, 1973 through June 30, 1974
Laboratory of Neuro-otolaryngology, IR
National Institute of Neurological Diseases and Stroke

Jorgen Fex, M.D., Ph.D., Chief

The Laboratory of Neuro-otolaryngology was established on April 10, 1974. Its chief entered on duty on November 15, 1974, as a Visiting Scientist. On January 20, 1974, a secretary was assigned to this Laboratory. On January 29, 1974, a technician entered on temporary duty not to exceed May 26, 1974.

SPACE

Ten modules have been allotted as space for the new Laboratory in adjoining Rooms 5D16-5D32 and Room 5D17 across the corridor. Plans have been submitted for complete remodeling of Rooms 5D24-5D32 and for partial remodeling of Rooms 5D22 and 5D17.

Room 5D24 will be used primarily for biochemistry, including high voltage electrophoresis. Rooms 5D26-5D30 will form one large laboratory for auditory neurophysiology; it will have an acoustic chamber in one corner for experiments that require good control over acoustic stimuli. It is expected that a second unit for physiological experiments may be set up outside the acoustic chamber, but still inside these three modules. Room 5D32 will be subdivided into two offices, for the chief of the Laboratory and for its secretary.

Room 5D17 was originally designed to be an animal room. However, it seems now a better policy to complete the remodeling of this room into laboratory space as well as that of Room 5D22, so that both these rooms can be made full use of for the biochemistry part of the program.

The Rooms 5D16-5D20 will be remodeled to serve the anatomy part of the program. In particular, a Philips 201C electron microscope will sit in a subdivision of Room 5D16. This room will also hold two darkrooms, one for processing photographic plates from the electron microscope and autoradiography and one for processing of other films and for printing.

The actual physical work for the remodeling had not been started at the time of the writing of this report, May 9, 1974, but is expected to begin at the end of the month. No prediction has been given for how long the work will take. The acoustic chamber in Rooms 5D26-5D30 and the high voltage electrophoresis equipment in Room 5D24 will be installed under separate contracts, probably immediately after July 1, 1974.

The plans for complete remodeling of the Rooms 5D16-5D20 and for the remaining partial remodeling of Rooms 5D22 and 5D17 will be drawn up during consultation with candidates for positions of anatomists and biochemists at the Laboratory; we expect to start on this during this month and we expect all ten rooms to be fully remodeled by the end of 1974.

PERSONNEL

No serious recruiting efforts could be made before an approximate timetable for the remodeling could be presented to candidates. Now, on May 9, the situation is the following:

For the Physiology Part of the Program:

1. The chief of the Laboratory.
2. A candidate for a position here as a Staff Fellow is to enter on duty on June 17 of this year. A request for his appointment is to be sent to the Director, NINDS.

   He received his Ph.D. in 1971. He is now doing postdoctoral experimental work on the auditory system at the Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, and at the Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts.

For the Biochemistry Part of the Program:

1. A candidate for a position here as a Staff Fellow is expected to enter on duty immediately after the oral examination for his Ph.D. at the end of August of this year, or as soon as it can be arranged through the National Institutes of Health Personnel Office for him to come. A request will leave this office within a week so that the first steps for his appointment may be taken.

   He has now a Training Grant at the Department of Chemistry, Indiana University, Bloomington, Indiana.

2. A second candidate for a position here as a Staff Fellow is expected to enter on duty as soon as he has received his Ph.D., about two years from now.

   He is at the same Department as the immediately preceding candidate and he has also a Training Grant.

3. A third candidate for a position as Staff Fellow will decide within a week whether he wants to come here. It is hoped that he will enter on duty by September 1 of this year.

   He received his Ph.D. in 1970 in biochemistry. He is now concerned with experimental studies on auditory physiology at the Central Institute for the Deaf, St. Louis, Missouri.

For the Anatomy Part of the Program:

1. A candidate for a GS-14 position will decide during this month whether he wants to come here. It is hoped that he will enter on duty during the spring of 1975.

He is Associate Professor, Department of Anatomy and Physiology, Indiana University, Bloomington, Indiana.

2. A second candidate for a position as Staff Fellow will also decide during this month whether to come. It is hoped that he will enter on duty in January, 1975.

   He became a Ph.D. in 1972 and is now a postdoctoral trainee, Department of Anatomy, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California.

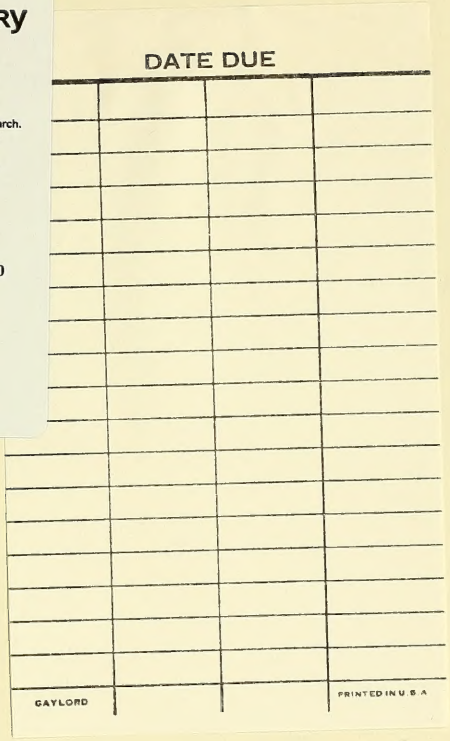
PROGRAM

The research of the new Laboratory will focus on defining inner ear mechanisms of mammals, using anatomical, biochemical and physiological techniques. It is expected that after a period of development the program of the new Laboratory will be concerned with the study of hereditary hearing disorders, aiming for findings that will lead to better understanding and management of such disorders in man.

Library, ... Unit
National ... of Health
Building 10
Bethesda, Maryland 20014

LIBRARY
NIH
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080



NIH LIBRARY
4 0129 1371

WERT
BOOKBINDING
MIDDLETOWN, PA.
DECEMBER 74
We're Quality Bound

NIH LIBRARY
3 1496 00186 8176